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(54) Title: METHOD OF SUPPRESSING β-AMYLOID-RELATED CHANGES IN ALZHEIMER'S DISEASE

(57) Abstract

Methods are provided for treating or preventing Alzheimer's disease comprising mucosally administering β-amyloid peptide or a fragment or analog thereof in an oral or other mucosal dosage form. Also disclosed herein are pharmaceutical formulation or dosage forms for use in these methods.

METHOD OF SUPPRESSING ß-AMYLOID-RELATED
CHANGES IN ALZHEIMER'S DISEASE

This application claims priority under 35 U.S.C.
§119 of U.S. application serial no. 60/067,219, filed
5   December 3, 1997 and U.S. application serial no. 60/079,697,
filed March 27, 1998, which applications are hereby
incorporated by reference in their entireties.

Field of the Invention

This invention relates to a treatment for
10 Alzheimer's disease based on mucosal tolerization.

Background of the Invention

1.   Alzheimer's Disease

Alzheimer's disease is the most common cause of
cognitive degeneration in the elderly.   Selkoe, D.J., et
15 al., Science, 235, 873-877 (1987). Extensive synaptic loss
occurs with resulting progressive memory loss, psychological
instability, decline in abstract reasoning, loss of bodily
function, coma, and ultimately death. Extracellular lesions
known as senile plaques develop in the brains of Alzheimer's
20 victims, comprising insoluble aggregates of β-amyloid (Aβ)
protein surrounded by dystrophic neurites. Mullan, M. and
Crawford, F., Trends Neurosci., 16, 398-403 (1993).   β-
Amyloid is a hydrophobic peptide comprising 39-43 amino acid
residues; it is characterized by a high tendency to form
25 aggregates, and results from proteolytic processing of a 90-
140 kDa integral membrane protein precursor known as amyloid
precursor protein (APP).

While formerly it was thought that β-amyloid
peptide itself was responsible for neurodegeneration in

Alzheimer's disease since the peptide can kill neurons _in_
vitro, it is now known that the APP precursor is expressed
in the brain cells of both healthy and Alzheimer's diseased
brains. Konig, G., et al., Mol. Brain Res., 9, 259-262

5   (1991). Hence, presence of the β-amyloid peptide is not by
itself sufficient to account for the disease.

Attention has recently shifted to the potential
involvement or participation in Alzheimer's of immunological
processes such as inflammation. A large body of research

10   evidence has accumulated showing certain parallels between
immunological events and Alzheimer's disease. Alzheimer's
brains display various structural features of immune-
mediated brain damage including increased populations of
microglial cells and astrocytes which express such

15   inflammatory cytokines and inflammation-associated agents as
interleukin-1 and α1-antichymotrypsin, and which are
immunoreactive with major histocompatibility complex (MHC)
class I and II. Abraham, et al., Cell, 52, 487 (1988).
Microglial cells are thought to be the functional equivalent

20   of macrophages in the central nervous system. Stryen, et
al., Exp. Neurol., 110, 93 (1990). Other features observed
in both immune-mediated and Alzheimer's brain damage include
immunoreactive markers such as MHC class I and II
glycoproteins, cytokines, complement receptors and

25   regulatory factors. McGeer, P.L., et al., Neurosci. Lett.,
107, 341-344 (1989); McGeer, P.L., et al., Brain Res., 544,
315-320 (1990); Griffin, W.S., et al., Proc. Nat'l. Acad.
Sci. U.S.A., 86, 7611-7615 (1989). Also, the inflammatory
mediator interleukin-6 (IL-6) which is found in Alzheimer's

30   brains is known to stimulate α-2-macroglobulin (α2M), a
highly potent human protease inhibitor produced by neurons
of Alzheimer's brains, and thought to interfere with normal
proteolytic processing of APP to form β-amyloid. Bauer, J.,
et al., FEBS Letts., 285, 111-114 (1991). The physiological

35   production of ß-amyloid protein and mechanism of Alzheimer's
disease is further described in Selkoe, Trends in
Neurosciences., 16:403-409 (1993).

It is also known that β-amyloid peptide present in insoluble form in Alzheimer's brain plaques can bind and activate the classical complement proteins (C1q) *in vitro* in the absence of antibody proteins (e.g., immunoglobulins).

5   In contrast, the soluble phase Aβ is subject to protective regulation by fluid-phase inhibitors, suggesting that it is the insoluble form of Aβ that is neurotoxic and explaining the apparent harmlessness of soluble Aβ. Rogers, *et al.*, Proc. Nat'l Acad. Sci. U.S.A., 89, 10016-10020 (1992).

10   McGeer and Rogers and their collaborators have proposed that aggregated β-amyloid may activate the complement cascade which leads to the generation of proteins capable of destroying neurons. *Cf.* Schnabel, J., New Scientist, 138, 22 (June 19, 1993). In further support of a mechanism link

15   between amyloid plaque deposition and immune response, it was found that microglial cells, the immune cells of the brain, can be activated with interferon-γ (IFN-γ) and [1-42]Aβ to produce significantly more damage to neurons than either stimulus alone. Meda, L., *et al.*, Nature, 374, 647-

20   650 (1995). This finding suggests a synergistic effect between Aβ and IFN-γ in triggering the generation by microglial cells of neurodegenerative substances such as reactive nitrogen intermediates (e.g., toxic free-radicals) and tumor necrosis factor-α (TNF-α). See, also Huberman, M.

25   et al., J. Neuroimmunol., 52:147-152, 1994 who report increased secretion in Alzheimer's patients of proinflammatory cytokines, and a correlation between cytokine levels and stage of the disease.

Epidemiological evidence also supports a nexus

30   between immunological activity and Alzheimer's disease. Anderson, K., *et al.*, Neurol., 45, 1441-1445 (1995). In the Rotterdam study surveying 7,983 subjects, non-steroidal anti-inflammatory drugs were shown to have a protective effect on the risk for developing Alzheimer's disease and an

35   ameliorating effect on the disease component of diminished cognitive function. In spite of these studies pointing to a role for the immune system in the induction of Alzheimer's

disease, no truly effective therapeutic regimes have emerged based on these research results.

Current treatments for Alzheimer's disease are generally few and inadequate. TACRINE™ (1,2,3,4-tetrahydro-9-acridinamine monohydrochloride monohydrate; also marketed as COGNEX™) is a reversible cholinesterase inhibitor with some efficacy for treating Alzheimer's disease. Watkins, P.B., et al., J. Amer. Med. Assoc., 271, 992 (1994). TACRINE has been only partially effective in community-dwelling subjects with mild or moderate dementia. Weiner, M.F., Consultant, 35, 313 (1995). Aside from its limited efficacy, there are safety concerns for its use. These include the elevation of serum aminotransferase levels which may indicate hepatocellular injury. Other dangers involve a risk of developing liver necrosis, jaundice or hepatitis which can be severe or fatal in particularly susceptible patients.

Other approaches have been attempted, such as the treatment of psychological symptoms of Alzheimer's disease using selective serotonin re-uptake inhibitors, e.g., paroxetine, fluoxetine, or trazodone, and the administration of cholinergic agonists or precursors, e.g., lecithin and nicotine but have met with similarly limited success. Pilot clinical studies suggest that nicotine may be useful to treat deficits in attention and information processing resulting from Alzheimer's disease. Sahakian, et al., Brit. J. Psych., 154, 797 (1989). Nicotine, however, is poorly bioavailable, and therefore not useful as a clinical treatment.

Viewing Alzheimer's disease as a chronic inflammatory state, McGeer and Rogers have proposed using anti-inflammatory agents such as indomethacin, a drug which curbs inflammation by blocking the action of cyclooxygenase, to treat the disease. McGeer, P.L., et al., U.S. Patent No. 5,192,753, issued March 9, 1993. In a study of 44 subjects with early-stage dementia, it was found that the group receiving the drug indomethacin did not deteriorate in mental function during the study. Schnabel, J., New

<u>Scientist</u>, *supra*. However, various side effects of indomethacin, including gastro-intestinal effects, make its clinical use problematic.

5   Garvey, *et al.*, U.S. Patent No. 5,409,946, issued April 25, 1995, disclose certain isoxazole, isothiazole and pyrazole compounds which allegedly enhance cognitive function, but do not appear to treat Alzheimer's disease directly.

       In general, all known methods of treating
10   Alzheimer's disease have met with no significant success. A clear need therefore exists for improved methods of treating the disease.

       2.   <u>Oral and, More Generally, Mucosal Tolerization</u>

       A mucosal tolerization approach to treating T-cell
15   mediated autoimmune diseases is discussed extensively in PCT International Application No. PCT/US93/01705. This method involves the oral or mucosal administration of antigens specific to the tissue under autoimmune attack which may or may not be themselves a target of the autoimmune attack
20   ("bystander antigens"). Oral or mucosal administration of these "bystander antigens" causes active suppression, i.e. the induction of regulatory (suppressor) T-cells in the gut-associated lymphoid tissue (GALT), or, in the case of by-inhalation administration, bronchial-associated lymphoid
25   tissue (BALT). See, also Miller, A., *et al.*, <u>J. Exp. Med.</u>, 174, 791-798 (1991). These regulatory suppressor T-cells are released in the blood or lymphatic tissue and then migrate to the organ or tissue afflicted with the autoimmune disease where they suppress autoimmune attack mounted
30   against the afflicted organ or tissue. The T-cells elicited by the bystander antigen (which recognize at least one epitope of the bystander antigen used to elicit them) are targeted to the locus of autoimmune attack where they mediate the local release of one or more immunomodulatory
35   cytokines, such as transforming growth factor beta (TGF-β) interleukin-4 (IL-4) or interleukin-10 (IL-10) which down-regulate immune response.

Of these, TGF-β is an antigen-nonspecific immunosuppressive factor in that it suppresses all immune attack phenomena in the vicinity of its release regardless of the antigen that triggers these phenomena. (However,

5   because oral tolerization with a bystander antigen causes the release of immunoregulatory substances only in the vicinity of autoimmune attack, no systemic immunosuppression ensues.)

IL-4 and IL-10 are also antigen-nonspecific

10   immunoregulatory cytokines. IL-4 in particular enhances Th2 response, *i.e.*, it acts on T-cell precursors and causes them to differentiate preferentially into Th2 cells. IL-4 also indirectly inhibits Th1 exacerbation. IL-10 is a direct inhibitor of Th1 responses.

15   After mucosally tolerizing mammals afflicted with an autoimmune disease, increased levels of TGF-β, IL-4 and IL-10 were observed in the gut associated lymphoid tissue (Chen, Y., *et al.*, Science, 265, 1237-1240 (1994)) and a concomitant decrease in cytokines associated with immune

20   system (and particularly T-cell activation), such as IL-2, IFN-γ, etc. Downregulation of inflammatory cytokines and upregulation of TGF-β, and IL-4 were observed in the brains of EAE rats following mucosal tolerization of those animals (Khoury *et al.*, J. Exp. Med., 176, 1355-1364 (1992)).

25   Naive T-cells differentiate into distinct populations defined by their cytokine secretion pattern and regulatory function. Th1 cells secrete predominantly interleukin-2 (IL-2) and IFN-γ and are involved in classic delayed-type hypersensitivity reactions, while Th2 cells,

30   which secrete predominantly IL-4 and IL-10, induce selected immunoglobulin secretion and can down-regulate Th1-mediated immune responses Mossmann, T. R., *et al.*, J. Immunol., 136, 2348-57 (1986); Romagnan, S., Annu. Rev. Immunol., 12, 227-57 (1994). Thus, cytokines secreted by T-cells after

35   activation qualitatively influence the nature of the immune response. Recent reports have suggested that the dominant factors determining the maturation of naive T-cells into either predominantly Th1 or Th2 cells are cytokines,

particularly IL-2 and IFN-γ for Th1 and IL-4 for Th2. Seder,
R. A., et al., Annu. Rev. Immunol., 12, 635-673 (1994).
Other factors include the dose and type of antigen, the type
of antigen-presenting cell (APC), and co-stimulatory

5   molecules involved in activation. Once differentiated into
Th1 or Th2 type, T-cells were formerly thought to be
committed to the production of a given set of lymphokines
upon restimulation. However, it has been recently
discovered that certain MBP-specific, CD4⁺ T-cells, isolated

10   from the GALT of SJL mice orally tolerized with myelin basic
protein and MS patients orally tolerized with myelin and
structurally identical to Th1 disease-inducing clones in
terms of their T-cell receptor usage, MHC restriction and
epitope recognition, suppressed rather than induced disease

15   and may represent a novel T-cell subset (Th3) with both
mucosal T-helper function and down-regulatory properties
vis-a-vis Th1 and other immune cells.

         T-cell secretion of certain cytokines is also
implicated in the induction and regulation of autoimmune

20   inflammatory disease, which is mediated by activated
autoreactive T-cells that recognize self-tissue-specific
antigen in the context of MHC class II molecules on APCs.
In experimental autoimmune encephalomyelitis (EAE), a model
for multiple sclerosis, it is believed that T-cell secretion

25   of IL-2, IFN-γ, and TNF mediates inflammation and tissue
damage, while the secretion of IL-4, IL-10, and TGF-β1 by
myelin basic protein (MBP)-reactive T-cells is associated
with potent suppressor activity and down-regulation of
central nervous system inflammation. Miller, A., et al.,

30   Proc. Nat'l Acad. Sci. U.S.A., supra (1992). The induction
of anti-inflammatory cytokines, including TGF-β1, appears to
be of particular importance in regulating EAE. For example,
anti-TGF-β1 monoclonal antibodies inhibit the suppressor
effect of regulatory MBP-reactive T-cells. Chen, Y., et

35   al., Science, supra (1994). Thus, autoreactive T-cells are
not necessarily pathologic and can function to down-regulate
immune responses associated with tissue inflammation
locally. Similar findings have been made in humans

afflicted with multiple sclerosis. TNF has been isolated
from central nervous system plaques of MS patients, IFN-γ
exacerbates attacks, and TGF-β and/or IL-4 and/or IL-10 is
secreted by T-cells of MS patients tolerized with myelin.

5   Objects of the Invention
It is an object of the present invention to
provide novel immunomodulatory methods for treating
individuals suffering from Alzheimer's disease and for
preventing the disease in individuals at risk for
10   contracting the disease.
Another object of the present invention is to
provide compositions and pharmaceutical formulations useful
for preventing the induction of Alzheimer's disease in
animal models and for treating mammals suffering from the
15   disease.
It is a further object of this invention to devise
methods and compositions for suppressing certain cytokine
responses associated with Alzheimer's disease in an
individual by a mucosal mode of administration, and for
20   enhancing certain other cytokine responses associated with
the suppression of inflammatory responses associated with
the disease.
It is another object of the invention to prevent
deposition of ß-amyloid.
25   These and other objects of the present invention
will be apparent to those of ordinary skill in the art in
light of the following.

Summary of the Invention
It has been unexpectedly discovered by the present
30   inventors that mucosal administration of an antigen
associated with ß-amyloid plaques (such as β-amyloid peptide
and fragments thereof) is an effective treatment for disease
associated with said plaques. In one aspect, the invention
is directed to a method for treating Alzheimer's disease in
35   a mammal in need of such treatment, comprising orally (or
more generally, mucosally) administering to the mammal an
effective amount of an agent comprising whole β-amyloid

peptide or an effective fragment of β-amyloid peptide,
particularly fragments recognized by T-cells of the host
(including, without limitation, the peptides [1-40]Aβ, [1-
42]Aβ and [25-35]Aβ). Preferably, the administration is
5   continued for a period of time sufficient to achieve at
least one of the following:

    (i)   reduction in the level of one or more
                   proinflammatory Th1 cytokines;

    (ii)   increase in the level of one ore more
10   anti-inflammatory Th2 or Th3 cytokines;

    (iii)   amelioration, retardation or suppression
                   of at least one clinical or histological
                   symptom of Alzheimer's disease; or

    (iv)   suppression of ß-amyloid deposition.
15   Mucosally administered β-amyloid peptide according to the
invention can initiate immunological responses in a subject
which prevent, retard or arrest neuron cell destruction
and/or prevent deposition of ß-amyloid in neurons.

     The present invention also relates to formulations
20   adapted for mucosal administration, and/or delivery systems
adapted for mucosal administration, comprising an antigen
associated with Alzheimer's brain plaques (such as β amyloid
peptide or a fragment thereof) and useful in the treatment
of Alzheimer's disease. These and other embodiments of the
25   subject invention will be described in the detailed
description.

     In another aspect of the invention, ß-amyloid
peptide (or an effective fragment or analog thereof) is
mucosally administered to treat amyloid plaque formation
30   associated with Down's syndrome, Hereditary Cerebral
Hemorrhage with Amyloidosis-Dutch Type (HCHWA-D), sporadic
and familial ß-amyloid angiopathy, or other diseases
involving cerebral accumulation of ß-amyloid.

Brief Description of the Drawings

35   Figure 1A illustrates proliferation (expressed as
Stimulation Index (S.I.)) of T-cells from spleens of control
and experimental SWISSXBDF1 mice either fed (75 μg/feeding x

5) or nasally treated (25 μg/application x 3) with [1-40]β-
amyloid peptide and immunized two days after the last
treatment with 100 μg [1-40]β-amyloid peptide in 50 μg CFA
per mouse. Proliferation was determined ten days after
5   immunization.

      Figure 1B illustrates proliferation (expressed as
S.I.) of popliteal lymph node T-cells from control and
experimental SWISSXBDF1 mice and mice fed or nasally
administered [1-40]β-amyloid peptide determined ten days
10   after being immunized in the foot pad with 100 μg [1-40]β-
amyloid peptide in 50 μg CFA per mouse. Control mice
(designated "non") were only immunized.

      Figure 2A illustrates proliferation (expressed as
ΔCPM relative to nonimmunized mice) of T-cells from
15   popliteal lymph nodes from SJL/J, DBA/1, C57BL/6, BALB/C and
C3H/EB mice immunized with [1-40]β-amyloid peptide but not
fed any peptide.

      Figure 2B illustrates proliferation (expressed as
ΔCPM as in Fig. 2A) of spleen T-cells from SJL/J, DBA/1,
20   C57BL/6, BALB/C and C3H/EB mice immunized with [1-40]β-
amyloid peptide but not fed any peptide.

      Figure 3A illustrates the secretion (expressed as
Δpg/ml relative to nonimmunized controls) of cytokines IL-2,
IFN-γ, IL-4, IL-10 and TGF-β in popliteal lymph node T-cells
25   from SJL/J, DBA/1, C57BL/6, BALB/C and C3H/EB mice immunized
with [1-40]β-amyloid peptide but not fed any peptide.

      Figure 3B illustrates the secretion (expressed in
Δpg/ml as in Fig. 3A) of cytokines IL-2, IFN-γ, IL-4, IL-10
and TGF-β in T-cells isolated from spleens of SJL/J, DBA/1,
30   C57BL/6, BALB/C and C3H/EB mice immunized with [1-40]β-
amyloid peptide but not fed any peptide.

      Figure 4A illustrates the secretion (expressed as
in Fig. 3A) of cytokine IL-10 in T-cells from popliteal

lymph nodes of C3H/EB mice either fed (5, 50 or 500
μg/feeding for 5 days) or nasally administered (5 or 50
μg/application for 3 days) [1-40]β-amyloid peptide prior to
immunization (2 days after the last feeding or nasal

5   application) with 100 μg [1-40]β-amyloid peptide in 50 μg
CFA.

      Figure 4B illustrates the secretion (expressed as
in Fig. 3A) of cytokine IL-10 in T-cells from spleens of
C3H/EB mice either fed (5, 50 or 500 μg/feeding for 5 days)

10   or nasally administered (5 or 50 μg/application for 3 days)
[1-40]β-amyloid peptide prior to immunization (2 days after
the last feeding or nasal application) with 100 μg [1-40]β-
amyloid peptide in 50 μg CFA.

      Figure 5A illustrates the secretion (expressed as

15   in Fig. 3A) of cytokine IL-2 in popliteal lymph node T-cells
from C3H/EB mice either fed (5, 50 or 500 μg/feeding for 5
days) or nasally administered (5 or 50 μg/application for 3
days) [1-40]β-amyloid peptide prior to immunization (2 days
after the last feeding or nasal application) with 100 μg [1-

20   40]β-amyloid peptide in 50 μg CFA.

      Figure 5B illustrates the secretion (expressed as
in Fig. 3A) of TGF-β cytokine IL-2 in T-cells from spleens
of C3H/EB mice either fed (5, 50 or 500 μg/feeding for 5
days) or nasally administered (5 or 50 μg/application for 3

25   days) [1-40]β-amyloid peptide prior to immunization (2 days
after the last feeding or nasal application) with 100 μg [1-
40]β-amyloid peptide in 50 μg CFA.

      Figure 6A illustrates the secretion (expressed as

30   in Fig. 3A) of TGF-β in popliteal lymph node T-cells from
C3H/EB mice either fed (5, 50 or 500 μg/feeding for 5 days)
or nasally administered (5 or 50 μg/application for 3 days)
[1-40]β-amyloid peptide prior to immunization (2 days after
the last feeding or nasal application) with 100 μg [1-40]β-

35   amyloid peptide in 50 μg CFA.

Figure 6B illustrates the secretion (expressed as in Fig. 3A) of TGF-β in T-cells from spleens of C3H/EB mice either fed (5, 50 or 500 μg/feeding for 5 days) or nasally administered (5 or 50 μg/application for 3 days) [1-40]β-amyloid peptide prior to immunization (2 days after the last feeding or nasal application) with 100 μg [1-40]β-amyloid peptide in 50 μg CFA.

Figure 7A illustrates the secretion (expressed as in Fig. 3A) of IFN-γ from popliteal lymph node T-cells from SWISSXBDF1 mice fed 0 or 75 mg [1-40]β-amyloid peptide 5 times or nasally administered 25 mg of the same peptide 3 times.

Figure 7B illustrates the secretion (expressed as in Fig. 3A) of IL-2, IL-10, IL-4 and TGF-β from T-cells as described for Figure 7A.

Figure 8A illustrates the secretion (expressed as in Fig. 3A) of IFN-γ from T-cells as described in Fig. 6B.

Figure 8B illustrates the secretion of IL-2, IL-4, IL-10 and TGF-β from T-cells as described in Fig. 6B.

Figures 9A and 9B list results from the assessment of plaque burden, cytokine secretion, and inflammatory markers based on examination of brain sections obtained from PDAPP mice that were orally or nasally administered [1-40] β-amyloid.

Figure 10 shows P values calculated using the 1-tailed Mann-Whitney U-test for various group comparisons among the PDAPP mice that were orally or nasally administered [1-40] β-amyloid.

Figure 11 shows results of immunoassays conducted on mouse antisera obtained from PDAPP mice to determine the presence of various subtypes of antibodies to β-amyloid.

Figure 12 is a bar graph showing results of *in vitro* experiments in which PLN from PDAPP mice that were immunized with [1-40] β-amyloid peptide prior to sacrifice were tested for production of IL-2.

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Figure 13 is a bar graph showing results of *in vitro* experiments in which PLN from PDAPP mice that were immunized with [1-40] β-amyloid peptide prior to sacrifice were tested for proliferation in the presence of [1-40] β-

10   amyloid peptide.

Figure 14 is a bar graph showing results of *in vitro* experiments in which PLN from PDAPP mice that were immunized with [1-40] β-amyloid peptide prior to sacrifice were tested for production of IFN-γ.

15   Figure 15 is a bar graph showing results of *in vitro* experiments in which PLN from PDAPP mice that were immunized with [1-40] β-amyloid peptide prior to sacrifice were tested for production of IL-6.

20   Figure 16 is a bar graph showing results of *in vitro* experiments in which PLN from PDAPP mice that were immunized with [1-40] β-amyloid peptide prior to sacrifice were tested for production of IL-10.

Figure 17 is a bar graph showing results of *in vitro* experiments in which PLN from PDAPP mice that were
25   immunized with [1-40] β-amyloid peptide prior to sacrifice were tested for production of TGF-β.

Figure 18 shows results from an experiment in
30   which [1-40] β-amyloid was nasally administered to (B6D2)F1 mice in conjunction with nasal administration of IL-4 or IL-10, and prior to immunization of the mice with [1-40] β-amyloid. The Figure shows proliferation *in vitro* of spleen

cells from the mice in the presence of the [1-40] β-amyloid peptide.

        Figure 19 shows results from an experiment in
which [1-40] β-amyloid was nasally administered to (B6D2)F1
mice in conjunction with nasal administration of IL-4 or IL-
10, and prior to immunization of the mice with [1-40] β-
amyloid. The Figure shows production of IFN-γ *in vitro* in
spleen cells from the mice in the presence of the [1-40] β-
amyloid peptide.

        Figure 20 shows results from an experiment in
which [1-40] β-amyloid was nasally administered to (B6D2)F1
mice in conjunction with nasal administration of IL-4 or IL-
10, and prior to immunization of the mice with [1-40] β-
amyloid. The Figure shows production of IL-6 *in vitro* in
spleen cells from the mice in the presence of the [1-40] β-
amyloid peptide.

        Figure 21 shows results from an experiment in
which [1-40] β-amyloid was nasally administered to (B6D2)F1
mice in conjunction with nasal administration of IL-4 or IL-
10, and prior to immunization of the mice with [1-40] β-
amyloid. The Figure shows production of IL-10 *in vitro* in
spleen cells from the mice in the presence of the [1-40] β-
amyloid peptide.

        Figure 22 shows results from an experiment in
which [1-40] β-amyloid was nasally administered to (B6D2)F1
mice in conjunction with nasal administration of IL-4 or IL-
10, and prior to immunization of the mice with [1-40] β-
amyloid. The Figure shows production of TGF-β *in vitro* in
spleen cells from the mice in the presence of the [1-40] β-
amyloid peptide.

Detailed Description of the Invention

All publications and patent applications listed herein are hereby incorporated by reference.

The following terms as used in this disclosure have the meanings ascribed to them below.

"Mammal" is defined herein as any warm-blooded higher vertebrate organism (including a human) having an immune system and being susceptible to Alzheimer's disease or an induced or spontaneous animal model thereof.

"β-Amyloid peptide" is intended to include a peptide fragment of between about 10 to 43 (preferably 13-43) amino acid residues, the sequence of which is derived from β-amyloid precursor protein, prepared either by chemical synthetic methods known in the peptide art, including enzymatic methods or biosynthetic methods such as by isolation of peptide fragments from any source, including native tissue cultures, or by recombinant genetic engineering methods. Nonlimiting examples of β-amyloid peptide fragments encompassed by the present method and formulations include [1-40]Aβ, [1-42]Aβ, [25-35]Aβ, and analogs thereof containing substantially similar sequences and homologous, non-peptidic or isosteric replacements for selected residues. Effective peptide fragments include those having the property of suppressing a proinflammatory Th1 cytokine, decreasing the quantity of an inflammatory marker; increasing the quantity of an anti-inflammatory Th2 or Th3 cytokine, or suppressing ß-amyloid deposition. Homologous replacements entail amino acid residues of related structure and differing only in the number of methylene groups present in the residue. Non-peptidic replacements include organic structures with conformational properties similar to those natural amino acid residues. Isosteric replacements refer to residues with either single atom substitutions (e.g., S for O in the aromatic hydroxyl in tyrosine) or substitutions of one moiety for another occupying a similar volume of space (e.g., an amino group for a methyl group). The useful ß-amyloid peptides administered according to the invention include, among others, those having the sequences of human, bovine, and mouse ß-amyloid. It is preferred to administer to humans a

purified Aβ peptide having the amino acid sequence of the
human or bovine Aβ peptide [1-43] or a fragment thereof
recognized by the T-cells of the host that suppress
autoimmune reaction associated with Alzheimer's disease.

5   The peptide can form part of a longer amino acid sequence as
long as the T-cell recognition site is not disturbed.

"Abatement", "suppression" or "reduction" of an
immune response or reaction includes partial reduction or
amelioration of one or more symptoms of the attack or

10   reaction, e.g., reduction in number of activated T-cells or
in number of antibodies or in the levels of at least one
proinflammatory cytokine (e.g. γ-IFN, IL-2, IL-6, or TNF) or
an increase in the levels of at least one anti-inflammatory
cytokine, such as TGF-β, IL-4, IL-10.

15   "Treatment" of Alzheimer's disease is intended to
include, although not be limited to, one or more of the
following:

(i)   alteration of the profile of one or more
cytokines or inflammatory markers in an Alzheimer's patient

20   or animal model so that it conforms to or approaches the
cytokine profile of a sex-and-age-matched subject without
cognitive impairment;

(ii)   a delay in the progress of Alzheimer's
related dementia as evaluated by at least one of cognitive

25   impairment and other NINCDS-ADRFA criteria (McKhan et al.
1984) including without limitation blood count, blood
chemistry, Vitamin B12, Folic Acid, VDRL, thyroid function,
electroencephalogram, computed tomography, or criteria
according to the Record for Independent Living.

30   (iii) an amelioration of dementia as evaluated by
one or more of the symptoms, factors and criteria detailed
in item (ii) of this definition, or by observation by a
physician specializing in Alzheimer's disease, or

(iv) a decrease in the amount of ß-amyloid

35   associated plaque than would otherwise have been observed
absent the treatment.

Mucosal tolerance according to the invention is an
advantageous method for treating Alzheimer's disease,
dysfunction for at least the following reasons:

(1) Absence of toxicity. For example, no toxicity has been observed in clinical trials or animal experiments involving oral or other mucosal administration of other protein antigens, such as bovine myelin (which contains MBP and PLP) to humans afflicted with multiple sclerosis, or oral or by-inhalation administration of chicken Type II collagen to humans or rodents afflicted with rheumatoid arthritis (or a corresponding animal model disease); or oral administration of bovine S-antigen to humans afflicted with uveoretinitis; or oral administration of insulin to healthy volunteers.

(2) Convenience of therapy. Mucosal administration is more convenient than parenteral, or other forms, of administration.

The present inventors carried out a series of experiments to discover whether mucosal tolerization techniques causes suppression of β-amyloid-related inflammation characteristic of Alzheimer's disease. The use of the mucosal route to induce tolerance against an autoimmune response was found effective in an animal model to suppress undesirable immune responses associated with Alzheimer's disease, and to reduce histological symptoms of the disease in an animal model. Antigen feeding was implemented in test animals as a means of generating peripheral tolerance, and was successful in tolerizing Th1 type responses, while increasing the response of Th2 and Th3 lymphocytes, including increased anti-inflammatory cytokine responses as well as decreased popliteal lymph node proliferation in C3H/eb and DBA/1 mice. In a mouse model of Alzheimer's disease that simulates β-amyloid deposition, the PDAPP model, anti-inflammatory Th1 type responses were suppressed by mucosal administration of β-amyloid.

The present method of treating Alzheimer's disease based on the mucosal administration results, in one embodiment, in regulation of the specific immune response thought to be associated with the disease. While not wishing to be bound by theory, the present inventors believe that suppression of this response is effected primarily by an active suppression mechanism, i.e., the elicitation of T-

cells characterized by an anti-inflammatory cytokine
profile. Also, as discussed below, the method of the
invention has been associated with induction of antibodies
of β-amyloid which, without wishing to be bound by any
5   theory of the invention, may have a role in the beneficial
results observed.

As shown in the results described below, the
present method can also result in suppression of the
deposition of ß-amyloid. The experiments described in the
10 Examples show that ß-amyloid deposition was suppressed in a
mouse Alzheimer's disease model.

"Mucosal" administration includes oral, enteral,
intragastric, nasal, buccal or intrapulmonary
administration, and more generally any method of
15 administration (e.g. by inhalation) of an active ingredient
that brings the ingredient in contact with the immune system
of the treated subject at the mucosa-associated lymphoid
tissue, (MALT), including that of the gut, nasal, buccal,
bronchial or pulmonary mucosa. In one embodiment of the
20 invention, an oral composition administered according to the
invention is directed primarily to the gastric mucosa; this
is accomplished, for example, in a dosage form that does not
substantially administer the active ingredient to the oral
mucosa. Also, in experiments described below,
25 administration by inhalation has been found to be
particularly effective.

In one embodiment, the present invention provides
a method for treating a mammal suffering from (or at risk
for developing) Alzheimer's disease comprising mucosally
30 administering to the mammal an effective amount of a
composition comprising a β-amyloid peptide, e.g. [1-40]ß-
amyloid, [1-42]β-amyloid, [25-35]β-amyloid or other β-
amyloid peptide greater than about 10 amino acids in length.
In one embodiment, the β-amyloid peptide sequence is greater
35 than 15 amino acids in length, and in another greater than
20 amino acids in length. Administration is preferably
continued for a period of time sufficient to achieve a
change in one of the parameters described above. In a

preferred embodiment, the present invention provides the method as described wherein the mammal is a human.

The present invention also provides a pharmaceutical formulation for administration to a mammal

5   suffering from Alzheimer's disease comprising an oral or other mucosal dosage form and delivery system containing an effective amount of a composition comprising a β-amyloid peptide that is sufficient to achieve at achieve one of the above-described measures of treatment.

10   In one embodiment, the present invention provides the above-described pharmaceutical formulation wherein the oral dosage form is a solid dosage form selected from the group consisting of a tablet, a capsule and a caplet. As noted above, a preferred embodiment of the oral dosage form

15   of the invention delivers the composition comprising the β-amyloid peptide primarily to the gastric mucosa. Such an embodiment includes a tablet, capsule, caplet or other dosage form that avoids substantial dissolution in the mouth, e.g., where less than 10%, preferably less than 5%,

20   of the active ingredient in the dosage form is released in the mouth. In another embodiment, the present invention provides the pharmaceutical formulation as described above wherein the oral dosage form comprises an aqueous suspension solution of β-amyloid peptide. In additional embodiments,

25   the present invention provides the pharmaceutical formulation as above-described further comprising a pharmaceutically acceptable carrier or diluent.

The present invention also provides a pharmaceutical formulation for administration to a mammal

30   suffering from Alzheimer's disease comprising a dosage form according to the invention adapted for nasal or bronchial administration. In one preferred embodiment, the present invention provides the pharmaceutical formulation in aerosol or spray form to be delivered by inhalation as described

35   above further comprising a pharmaceutically acceptable carrier or diluent. The formulation can, for example, be administered in a nebulizer or inhaler. Such dosage forms are used to achieve the particularly beneficial results that

have been determined by the inventors to be associated with
administration by inhalation, e.g, by nasal administration.

Antigens that May be Used to Induce Tolerance

Suitable antigens for use in the invention include
5   antigens specific for Alzheimer's disease, i.e. antigens
which are recognized by immune T-cells of a human or animal
host. Non-limiting examples include synthetic or
biosynthetic β-amyloid peptide, including peptide fragments
thereof containing between 10 and 43 residues, preferably
10   between 13 and 43 residues. Examples of such antigens
include the β-amyloid peptides [1-40]Aβ, [1-42]Aβ and [25-
35]Aβ. Encompassed within the scope of the present
invention are various related substances sharing at least
one such T-cell recognition site with β-amyloid peptide.
15   Such substances include analogs of β-amyloid peptide which
vary in length or composition, but containing substantially
similar sequences (i.e., possessing significant levels,
e.g., between about 40% and about 95%, of primary sequence
homology). Such substances also include peptidic constructs
20   of β-amyloid peptide (or aforementioned) analogs thereof and
one or more flanking amino acid sequence found in native β-
amyloid protein or of extra β-amyloid origin. The invention
includes administration of amyloid precursor protein, and of
homologs of that protein; examples are described in US
25   Patent Nos. 5,525,714, 5,441,931, and 5,436,153. Amyloid
precursor protein is also described in US Patent Nos.
5,318,958 and 5,218,100. cDNA clones encoding amyloid are
also described in US Patent No. 4,912,206. The invention
also encompasses peptides having conservative amino acid
30   substitutions with respect to naturally occurring ß-amyloid
peptides, e.g., having one or more, preferably less than
five, conservative substitutions with respect to the human,
bovine, or mouse sequences. Methods for assessing the
effectiveness of such β-amyloid peptides, β-amyloid peptide
35   analogs and constructs sharing at least one T-cell
recognition site with a β-amyloid peptide are well-known in
the art. For example, the overlapping peptide method can be
used to test various fragments (having at least 10 and

preferably 20 amino acid residues) of β-amyloid peptide Aβ
[1-39] (or Aβ [1-43] as the case may be) by placing them in
contact with a preparation containing immune T-cells
isolated from a host suffering from Alzheimer's disease and
5   assessing the proliferation of such T-cells. Additionally,
the cytokine patterns of such T-cells can be analyzed using
the methodology provided below in the examples to further
refine choice of a peptide. In one embodiment, the peptide
is one which causes T-cells secreting anti-inflammatory
10 cytokines to proliferate. In another embodiment, the
peptide is one which causes suppression of one or more
inflammatory responses, whether or not it causes T-cells
secreting anti-inflammatory cytokines to proliferate or
otherwise increases production of such anti-inflammatory
15 cytokines. Peptides are also encompassed that suppress ß-
amyloid deposition, as shown, e.g., in the mouse model
described in the Examples. In another embodiment, peptides
are encompassed which cause an increase in the quantity of
anti-β amyloid antibodies present in sera, e.g., in the sera
20 of a human or non-human host suffering from, or predisposed
to, Alzheimer's disease or a disease model of Alzheimer'.s
disease. Peptides are also encompassed that have the
sequence of a β-amyloid peptide, but including one, two, or
three altered contact points for MHC or T-cell receptor, as
25 determined by methods known in art using cells obtained from
Alzheimer's patients.

Formulations for Mucosal Administration

Administration of more than one antigen is
possible, and may be desirable (e.g. when the patient's
30 immune T-cells recognize more than one antigen).

Suitable formulations according to the invention
include formulations adapted for oral, enteral, buccal,
nasal, bronchial or intrapulmonary administration. The
preparation of such formulations is well within the skill of
35 the art. It is preferred that the antigens employed be of
synthetic provenance and not isolated from biological
sources to avoid the risk of infection (notably, but not
exclusively, to avoid transmission of agent responsible for

the Creutzfeld-Jacob disease). Additionally, it is preferred that the formulation not contain adsorption promoting agents or ingredients that protect against proteolytic degradation. As further described below, however, the antigens can be combined with one ore more enhancing agents, such as IL-4 or IL-10, to increase the effectiveness of the formulation.

Suitable oral formulations for use in tolerization of T-cell mediated immune responses according to the present invention can be in any suitable orally administrable form, for example, a pill, a liquid, or a capsule or caplet containing an effective amount of antigen. Each oral formulation may additionally comprise inert constituents including pharmaceutically acceptable carriers, diluents, fillers, disintegrants, flavorings, stabilizers, preservatives, solubilizing or emulsifying agents and salts as is well-known in the art. For example, tablets may be formulated in accordance with conventional procedures employing solid carriers and other excipients well-known in the art. Capsules may be made from any pharmaceutically acceptable materials, such as gelatine or cellulose derivatives. Nonlimiting examples of solid carriers include starch, sugar, bentonite, silica and other commonly used inert ingredients. Diluents for liquid oral formulations can include *inter alia* saline, syrup, dextrose and water.

The antigens used in the present invention can also be made up in liquid formulations or dosage forms such as, for example, suspensions or solutions in a physiologically acceptable aqueous liquid medium. Such liquid media include water, or suitable beverages, such as fruit juice or tea which will be convenient for the patient to sip at spaced apart intervals throughout the day. When given orally in liquid formulations the antigen may be dissolved or suspended in a physiologically acceptable liquid medium, and for this purpose the antigen may be solubilized by manipulation of its molecule (e.g., hydrolysis, partial hydrolysis or trypsinization) or adjustment of the pH within physiologically acceptable limits (e.g. 3.5 to 8). Alternatively, the antigen may be

reduced to micronized form and suspended in a physiological-
ly acceptable liquid medium, or in a solution.

Sustained release oral delivery systems are also
contemplated and are preferred. Nonlimiting examples of
sustained release oral dosage forms include those described
in U.S. Patent No. 4,704,295, issued November 3, 1987; U.S.
Patent No. 4,556,552, issued December 3, 1985; U.S. Patent
No. 4,309,404, issued January 5, 1982; U.S. Patent No.
4,309,406, issued January 5, 1982; U.S. Patent No.
5,405,619, issued April 10, 1995; PCT International
Application WO 85/02092, published May 23, 1985; U.S. Patent
No. 5,416,071, issued May 16, 1995; No. 5,371,109 issued
December 6, 1994; U.S. Patent No. 5,356,635, issued Oct. 18,
1994; U.S. Patent No. 5,236,704, issued August 17, 1993;
U.S. Patent No. 5,151,272, issued September 29, 1992; U.S.
Patent No. 4,985,253, issued January 15, 1991; U.S. Patent
No. 4,895,724, issued January 23, 1990; and U.S. Patent No.
4,675,189, issued June 23, 1987.

Sustained release oral dosage forms coated with
bioadhesives can also be used. Examples are compositions
disclosed in EP 516,141; U.S. Patent No. 4,226,848, Oct.
1980; U.S. Patent No. 4,713,243; U.S. Patent No. 4,940,587;
PCT International Application WO 85/02092; EPO 0 205 282;
Smart, *et al.*, J. Pharm. Pharmacol., **36**, 295-99 (1984);
Sala, *et al.*, Proceed. Intem. Symp. Control. Rel. Bioact.
Mater., **16**, 420-21 (1989); Hunter, *et al.*, International
Journal of Pharmaceutics, **17**, 59-64 (1983); "Bioadhesion -
Possibilities and Future Trends, Kellaway," Course No. 470,
May 22-24, 1989.

Commercially available sustained release
formulations and devices include those marketed by ALZA
Corporation, Palo Alto, CA, under tradenames ALZET, INFUSET,
IVOS, OROS, OSMET, or described in one or more U.S. Patents:
No. 5,284,660, issued Feb. 9, 1994; No. 5,141,750, issued
Aug. 25, 1992; No. 5,110,597, issued May 5, 1992; No.
4,917,895, issued April 17, 1990; No. 4,837,027, issued June
6, 1989; No. 3,993,073, issued Nov. 23, 1976; No. 3,948,262,
issued April 6, 1976; No. 3,944,064, issued March 16, 1976;

and No. 3,699,963; International Applications PCT/US93/10077 and PCT/US93/11660; and European Published Applications EP 259013 and EP 354742.

Sustained release compositions and devices are suitable for use in the present invention because they serve to prolong contact between the antigen and the gut-associated lymphoid tissue (GALT) and thus prolong contact between the antigen and the immune system. In addition, sustained release compositions obviate the need for discrete multi-dose administration of the antigen and permit the required amount of antigen to be delivered to GALT in one or two daily doses. This is anticipated to improve patient compliance.

Orally administrable pharmaceutical formulations containing one or more antigens such as β-amyloid peptides are prepared and administered to mammals who have manifested symptoms of Alzheimer's disease. Additionally, subjects who are at risk for developing Alzheimer's disease, i.e., have a genetic predisposition to developing the disease, as determined through suitable means, such as genetic studies and imaging analysis of the brain, are treated with similar oral preparations of β-amyloid peptide.

Pharmaceutical formulations for oral or enteral administration to treat Alzheimer's disease are prepared from a composition comprising a β-amyloid peptide and a pharmaceutically acceptable carrier suitable for oral ingestion. The quantity of β-amyloid peptide may be between 0.01 mg and 1000 mg per day. In one embodiment of the invention, the dose is greater than 0.05 mg per day. In another embodiment the dose is greater than 0.1 mg per day, and may be greater than 1 mg per day. In a further embodiment, the dose is between 0.05 and 1 mg per day. However, the total dose required for treatment can vary according to the individual and the severity of the condition. This amount can be further refined by well-known methods such as establishing a matrix of dosages and frequencies of administration.

For by-inhalation administration (i.e. delivery to the bronchopulmonary mucosa) suitable sprays and aerosols

can be used, for example using a nebulizer such as those
described in U.S. Patent Nos. 4,624,251 issued November 25,
1986; 3,703,173 issued November 21, 1972; 3,561,444 issued
February 9, 1971 and 4,635,627 issued January 13, 1971. The
5   aerosol material is inhaled by the subject to be treated.

Other systems of aerosol delivery, such as the
pressurized metered dose inhaler (MDI) and the dry powder
inhaler as disclosed in Newman, S.P. in <u>Aerosols and the
Lung</u>, Clarke, S.W. and Davia, D. eds. pp. 197-224,
10   Butterworths, London, England, 1984, can be used when
practicing the present invention.

Aerosol delivery systems of the type disclosed
herein are available from numerous commercial sources
including Fisons Corporation (Bedford, MA), Schering Corp.
15   (Kenilworth, NJ) and American Pharmoseal Co. (Valencia, CA).

Formulations for nasal administration can be
administered as a dry powder or in an aqueous solution.
Preferred aerosol pharmaceutical formulations may comprise
for example, a physiologically-acceptable buffered saline
20   solution containing the ß-amyloid antigen of the present
invention.

Dry aerosol in the form of finely divided solid ß-
amyloid particles that are not dissolved or suspended in a
liquid are useful in the practice of the present invention.
25   The antigen may be in the form of dusting powders and
comprise finely divided particles having an average particle
size of between about 1 and 5 um, preferably between 2 and 3
um. Finely divided antigen particles may be prepared by
pulverization and screen filtration using techniques well
30   known in the art. The particles may be administered by
inhaling a predetermined quantity of the finely divided
material, which can be in the form of a powder.

Specific non-limiting examples of the carriers
and/or diluents that are useful in the pharmaceutical
35   formulations of the present invention include water and
physiologically-acceptable buffered saline solutions such as
phosphate buffered saline solutions pH 7.0-8.0.

The nasally administered formulation of the
present invention may include a thermosetting gel which

increases in viscosity at body temperature upon contact with
the nasal mucosa.

Formulations for buccal administration can include
mucoadhesive mixed with effective amounts of the ß-amyloid
antigen. Effective amounts are anticipated to vary
according to the formulation employed. For formulation
administered by inhalation, the effective amount is likely
to be less than that of the oral dose.

The duration of treatment in humans should be a
minimum of two weeks, and typically three months, and may be
continued indefinitely or as long as benefits persist. The
treatment may be discontinued if desired (in the judgment of
the attending physician) and the patient monitored for signs
of relapse. If clinical symptoms or other disease
indicators show that the patient is relapsing, treatment may
resume.

As will be understood by those skilled in the art,
the dosage will vary with the antigen administered and may
vary with the sex, age, and physical condition of the
patient as well as with other concurrent treatments being
administered. Consequently, adjustment and refinement of
one or both of the dosages used and the administration
schedules will preferably be determined based on these
factors and especially on the patient's response to the
treatment. Such determinations, however, require no more
than routine experimentation, as illustrated in Examples
provided below.

Administration of β-amyloid antigens can be
conjoined with mucosal administration of one or more
enhancers, i.e. substances that enhance one or more
beneficial effect of the β-amyloid antigens. Such enhancers
include LPS, Lipid A (as described in U.S. Application
Serial No. 08/202,677, published as WO 91/01333) IL-4, IL-10
and Type I interferons, including α-interferon and β-
interferon (See, e.g. U.S. Application Serial No. 08/420,980
and 08/420,979 and WO 95/27499 and WO 95/27500), cholera
toxin B-subunit, and LTB (heat labile enterotoxin).
Experiments have been carried out, described further below,
in which administration of IL-4 in conjunction with β-

amyloid peptide was found to be particularly effective. As
used in this paragraph, "conjoined with" means before,
substantially simultaneously with, or after administration
of these antigens. Naturally, administration of the
5   conjoined substance should not precede nor follow
administration of the antigen by so long an interval of time
that the relevant effects of the substance administered
first have worn off. Therefore, enhancers should usually be
administered within about 24 hours before or after the β-
10   amyloid antigens and preferably within about one hour.

The invention is further described below by
reference to examples, the purpose of which is to illustrate
the present invention without limiting its scope. All
documents cited herein are incorporated by reference.

15   **Example 1**

Experimental Animals

Female BALB/C (6-8 weeks of age); DBA/2; C3H/eb;
C47b1/6; SWISSXBDF1 or SHL/J mice were used. The animals
were maintained in a temperature- and light-controlled
20   environment with free access to feed and water. Each
experimental group contained no fewer than 5 mice.

Immunization

Mice were immunized with 100 μg of [1-40]β-amyloid
peptide ([1-40]Aβ) emulsified in 50 μg CFA (Difco
25   Laboratories, Inc.). On day 10, popliteal lymph nodes and
spleens were removed and for T-cell proliferation and
cytokine production.

Proliferation Assay

Cells (5 x 10^5 per well) were cultured in
30   triplicate in 96-well round-bottomed plates (Falcon™, Becton
Dickinson, Lincoln Park, New Jersey) in the presence of
antigen (1-100 μg/ml) for 72 hr. [^{3}H]Thymidine (1 μCi/well)
was added for the last 7 hr of culture before harvesting the
cells. Incorporation of [^{3}H]thymidine was determined using a

Betaplate scintillation counter (Beckman Instruments Inc., Fullerton, California). The proliferation response was measured as Δcpm (cpm[test] - cpm[control without antigen]). The stimulation index (SI) was calculated as mean cpm[with

5   antigen]/mean cpm[without antigen].

Figure 1A shows significant suppression of proliferation of spleen cells from SWISSXBDF1 mice (nontransgenic litermates of Alzheimer's model) either fed (75 μg/feeding x 5) or nasally treated (25 μg/direct

10   application to nasal mucosa x 3) with [1-40]β-amyloid peptide and immunized two days after the last treatment with 100 μg [1-40]β-amyloid peptide in 50 μg CFA per mouse. Figure 1B shows suppression of proliferation in popliteal lymph nodes from SWISSXBDF1 mice either fed or nasally

15   treated with [1-40]β-amyloid peptide determined ten days after being immunized in the foot pad with 100 μg [1-40]β-amyloid peptide in 50 μg CFA per mouse. The results show that proliferation of spleen and popliteal lymph node T-cells to [1-40] β-amyloid peptide was significantly

20   suppressed in treated mice (fed or nasally administered the same peptide) compared to untreated controls. These results are particularly meaningful given that the cells tested for proliferative response were collected just 10 days after immunization, before the immunization effect subsided.

25   Figure 2A compares proliferation of popliteal lymph node T-cells from SJL/J, DBA/1, C57BL/6, BALB/C and C3H/EB mice immunized with [1-40]β-amyloid peptide. Figure 2B compares proliferation in spleen cells from SJL/J, DBA/1, C57BL/6, BALB/C and C3H/EB mice immunized with [1-40]β-

30   amyloid peptide. This experiment shows that many strains of normal mice (not transgenic mice, not Alzheimer's model) respond to [1-40]β-amyloid peptide, within a short time after immunization. It should be noted that T-cells for the experiment shown in this Example are stimulated with antigen

35   only once *in vitro* prior to the proliferation assay. Additional stimulations would enlist response from the apparent non-responder strain C57BL/6 and (DBA/1). This experiment provides information about the responding ability of cells from various strains and was useful in designing

the subsequent experiments described below. It is believed
to be advantageous to select "good responders" (such as
C3H/EB) but not "too high responders" (SJL/J was thus not
chosen).

<div align="center">

Example 2
</div>

Cytokine Production Assays

       Levels of IL-4, IL-2, IL-10, TGF-β and IFNγ in
supernatants were determined by capture ELISA as described.
Friedman, A., et al., _Proc. Nat'l Acad. Sci. U.S.A._, **91,**
6688 (1994); Yang, X., et al., _J. Immunol._, **150,** 4354
(1993). Briefly, supernatants were added to microtiter
(Maxisorp) plates, previously coated with rat anti-mouse IL-
4, IL-2, IL-10, TGF-β or IFN-γ monoclonal antibodies
(capture antibodies, Pharmingen; 1 μg/ml at 4°C over night)
and blocked with BSA-diluent/blocking solution (KPL).
Plates were washed, and standards and samples were added for
another overnight incubation at 4°C. Bound cytokine was
detected by the addition of ABTS (Kirkegaard & Perry).
Wells were washed, and cytokine levels determined using
biotinylated rat anti-mouse IL-4, IL-2, IL-10, TGF-β or IFN-
γ monoclonal antibodies (detecting antibodies, Pharmingen)
and a peroxide visualization system (peroxidase-labeled
streptavidin). IL-2 and IL-4 were analyzed after 24 hours
of cell incubation with antigen, IL-10 and IFN-γ after 40
hours and TGF-ß after 72 hours. Cytokine levels were
calculated from a log-log plot of absorbance vs.
concentration of recombinant cytokines (Pharmingen), and
results are expressed in pg/mL (for IL-4, IL-2, IL-10, TGF-β
and IFN-γ). Threshold sensitivities of ELISA assays were 5
pg/mL, 10 pg/mL and 2.5 ng/mL for IL-4, IL-2 and IFN-γ,
respectively.

Statistical analysis

       The statistical significance of differences
between experimental groups was determined using unpaired
two-tailed Student's t-test, with differences considered
significant at $P < 0.05$.

Determination that mice exposed to Aβ peptide either fed or

nasally administered display suppression of

immunostimulatory (proinflammatory) cytokines (e.g. IL-2 and

IFN-γ) and enhancement of immunoregulatory (anti-

5   inflammatory) cytokines (e.g. IL-4, IL-10 and TGF-β) in

vivo.

          C3H/eb mice were fed [1-40]Aβ 500 μg/feeding, 50
μg/feeding, or 5 μg/feeding every day for 5 days (200
μl/feeding). Nasal administration was given at 50
10   μg/application or 5 μg/application every day for 3 days (10
μl/application). Two days after the last feeding or nasal
administration, fed, nasal or non-fed mice were immunized in
the foot pad with 100 μg [1-40]Aβ in 50 μg CFA per mouse.
          Figure 3A provides a comparison of the secretion
15   of cytokines IL-2, IFN-γ, IL-4, IL-10 and TGF-β in popliteal
lymph nodes from SJL/J, DBA/1, C57BL/6, BALB/C and C3H/EB
mice immunized with [1-40]β-amyloid peptide but not fed or
otherwise treated with the peptide. Figure 3B compares the
secretion of these cytokines in spleen cells from a similar
20   range of mice strains immunized with [1-40]β-amyloid peptide
(also not treated with the peptide) Comparison of the
results in Fig. 3A and Fig. 3B shows that the T-cells from
spleens secrete a different cytokine profile from that of T-
cells from popliteal lymph nodes, and that the cytokine
25   profile varies with the strain of mouse. Again, the reason
for this experiment is to select responders that will show a
difference in the property tested under the experimental
conditions.
          Figure 4A shows the secretion of the
30   immunoregulatory cytokine IL-10 in popliteal lymph nodes
from C3H/EB mice either fed (5, 50 or 500 μg/feeding for 5
days) or nasally administered (5 or 50 μg/application for 3
days) [1-40]β-amyloid peptide prior to immunization (2 days
after the last feeding or nasal application) with 100 μg [1-
35   40]β-amyloid peptide in 50 μg CFA. Figure 4B demonstrates
the secretion of cytokine IL-10 in spleen cells from C3H/EB
mice either fed or nasally administered [1-40]β-amyloid
peptide under similar conditions. This experiment also

served as a preliminary dose response experiment for this mouse strain. Given that the T-cells underwent a single stimulation *in vitro* and that the effects of the

5   immunization (which would suppress IL-10 levels in all mice tested) had not subsided, the results, which show significant enhancement of IL-10 in at least one of spleen and PLN cells treated with at least one dose, provides a showing that the cytokine profile of T-cells that recognize [1-40]β amyloid peptide can be altered by tolerization to

10   the same antigen.

Figure 5A illustrates the result in secretion of the Th1 immunostimulatory (proinflammatory) cytokine IL-2 in popliteal lymph nodes from C3H/EB mice at various feeding levels (5, 50 and 500 μg/feeding for 5 days) and when

15   nasally administered (5 or 50 μg/application for 3 days) [1-40]β-amyloid peptide prior to immunization (2 days after the last feeding or nasal application) with 100 μg [1-40]β-amyloid peptide in 50 μg CFA. Figure 5B similarly shows the secretion of cytokine IL-2 in spleen cells from C3H/EB mice

20   either fed (at lower doses, e.g., 5 μg/feeding for 5 days) or nasally administered (at 50 μg/application for 3 days) [1-40]β-amyloid peptide prior to immunization (2 days after the last feeding or nasal application) with 100 μg [1-40]β-amyloid peptide in 50 μg CFA. The fact that IL-2 secretion

25   is suppressed at least at one dose of treatment in either the spleen or the PLN T-cells shows suppression of Th1 responses despite the nonoptimal (i) timing, of the experiment (relative to the time of immunization); (ii) amount fed; and (iii) stimulation of cells *in vitro* (only

30   once). Suppression of IL-2 secretion was clear by nasal administration (Fig. 5A) and at 5 μg fed and 50 μg nasal (Fig. 5B). Based on the results for Fig. 5A, and Figs. 5B, 6A, 6B, and 7A, 7B, it was decided to use 25 μg nasally and 75 μg orally in subsequent experiments. These are not

35   optimal amounts, but they provide a quantity for demonstrating differences within the experimental conditions employed. See Figs. 8A and 8B below.

Figure 6A shows secretion of TGF-β in popliteal lymph nodes from C3H/EB mice either fed (at lower doses; 5 or 50 μg/feeding for 5 days) or nasally administered (at 50 μg/application for 3 days) [1-40]β-amyloid peptide prior to

5   immunization (2 days after the last feeding or nasal application) with 100 μg [1-40]β-amyloid peptide in 50 μg CFA. Figure 6B illustrates the enhanced secretion of TGF-β in spleen cells from C3H/EB mice either fed (at lower dose; 5 and 50 μg/feeding for 5 days) or nasally administered (at

10   50 μg/application for 3 days) [1-40]β-amyloid peptide prior to immunization (2 days after the last feeding or nasal application) with 100 μg [1-40]β-amyloid peptide in 50 μg CFA.

Example 3

15   **Cytokine profiles after feeding or nasal administration and immunization of non-transgenic litermates of the transgenic mouse model of Alzheimer's disease.**

Feeding and Immunization: SWISSXXB6D2F1 mice were fed with [1-40]Aβ 75 μg/feeding x 5 or nasally administered

20   with 25 μg/application x 3, and 2 days after the last treatment, were immunized with 100 μg [1-40]Aβ in 50 μg CFA. Ten days later, the popliteal lymph node and spleen T-cells were checked for cytokine production as described above. The results of these experiments demonstrated that mice

25   pretreated with Aβ peptide and immunized with Aβ peptide had a reduced *in vitro* response to Aβ stimulation for

proinflammatory (Th1) cytokines (and IFN-γ and IL-2 at least pursuant to nasal administration of 25 μg) and an increased response for anti-inflammatory (Th2) cytokines (TGF-β and

30   IL-10). The absence of detected TGF-β in non-treated mice in Figure 8B is particularly significant. These observations confirmed studies disclosed in Examples 1-2 on normal mice of several strains showing increased anti-inflammatory cytokine responses as well as decreased PLN

35   proliferation in mice of certain strains (e.g., C3H/EB and DBA/1) which had been mucosally tolerized with Aβ peptide.

Example 4

Feeding, nasal administration and immunization of heterozygous PDAPP transgenic mice (animal model of Alzheimer's disease).

Transgenic PDAPP mice were acquired from Athena Neurosciences, Inc. (South San Francisco, CA). These mice express a human gene encoding a mutant form of ß-amyloid precursor protein and progressively develop ß-amyloid deposits in the hippocampus and cerebral cortex in a manner similar to that seen in Alzheimer's disease. The ß-amyloid deposits in the transgenic mice become associated with activated microglial cells, reactive astrocytes and other features of the periplaque inflammatory response seen in Alzheimer's disease brains.

4 to 5 month old PDAPP mice were fed or nasally administered either [1-40]ß-amyloid peptide or a control protein (myelin basic protein or ovalbumin in amounts of 50 or 500 μg per feeding). Administration was carried out three (nasal) or five times consecutively (oral) during an initial one week period, and then on a weekly basis until the animals were between 11 and 12 months old. The orally administered ß-amyloid peptide was in a dosage of 10 or 100 μg, and the nasally administered β-amyloid was in a dosage of 5 or 25 μg.

The feeding and nasal administration of β-amyloid peptide, or ovalbumin to PDAPP mice was conducted among the following mice groups:

Group 0:   Non treated; mice nos. 0.1-0.7

Group 1:   fed [1-40]ß-amyloid peptide 100μg/feeding; mice nos. 1.1-1.9

Group 2:   fed [1-40]ß-amyloid peptide 10μg/feeding; mice nos. 2.1-2.9

Group 3:   nasal [1-40]ß-amyloid peptide 25μg/treatment; mice nos. 3.1-3.9

Group 4:   nasal [1-40]ß-amyloid peptide 5μg/treatment; mice nos. 4.1-4.7

Group 5:   fed ovalbumin 500μg/feeding; mice nos. 5.1-5.6

Group 6:   nasal ovalbumin 50μg/treatment; mice nos. 6.1-6.6
Group 7:   fed MBP 500μg/feeding; mice nos. 7.1-7.5
Group 8:   nasal MBP 50μg/treatment; mice nos. 8.1-8.6

5   Two mice from each of groups 1, 5, and 8 were
immunized with β-amyloid peptide 10 days prior to sacrifice.

At the end of the treatment period, the mice were
sacrificed and brains, popliteal lymph nodes and spleens
removed. Brains were removed and immersed in 70% ethanol
10   fixative following by paraffin embedding and sectioning.
One hemisphere was frozen and cryostat-sectioned.
Thereafter, sections of the brains of the orally
treated mice were examined using antibodies and histological
stains. Antibodies to glial fibrillary acidic protein
15   (GFAP) were employed to show abnormal astrocytes, and
antibody to amyloid precursor protein employed to show
abnormal neurites. Lymphocytes were examined for cytokine
production *in vitro* in the presence of β-amyloid.
Results obtained in the examination of brain
20   sections are shown in Figure 9A and Figure 9B.
Specificially, results are shown for both visual and
computer assisted image analysis of β-amyloid plaque load
for the 8 experimental groups of mice that were tested;
values are shown for each mouse in the relevant group. The
25   computer analysis of plaque load represents the percent area
identified as plaque. The visual assessment of plaque load
was scored according to the table shown at the bottom of
Figure 9A.
These results demonstrated a difference in the
30   development of β-amyloid deposits in the mice treated with
β-amyloid as opposed to the control groups, with the
exception of certain ova treated groups, as discussed
further below, i.e., the treated groups developed fewer β-
amyloid deposits. The difference was statistically
35   significant for mice that were nasally administered 25 μg of
β-amyloid versus controls. P values calculated using the 1-
tailed Mann-Whitney U-test for various group comparisons are
shown in Figure 10.

Results of immunoreactivity tests conducted on the
sections are also shown in Figure 9A and 9B, showing
immunoreactivity (IR) of specific antibodies with cells
bearing the immunological markers, or secreting the

5   cytokines, shown. Results are shown in particular for the
presence of: Mac-1, a marker for activated antigen
presenting cells, such as microglia; F4/80, a marker for
antigen presenting cells; and MHC-II. Results are also shown
for immunoreactivity with the inflammatory cytokines IFN-γ,

10   TFN-α, and for the anti-inflammatory cytokines IL-4, IL-10,
and TGF-β. These data show that the mice treated mucosally
with β-amyloid exhibited decreased microglial activation in
the brain and decreased production of inflammatory
cytokines.

15   Results of immunohistochemistry experiments
conducted on mouse antisera are shown in Figure 11. In
these experiments, the antisera were tested in an
immunoassay for the presence of IgG subtypes against β-
amyloid. In the immunoassay, Down syndrome brain sections,

20   which contain large amounts of β-amyloid, were employed as
"capture" antigen. IgG in the mice antisera were bound to
the sections, and the presence of IgG subtypes in the bound
antibodies distinguished by secondary (2^0) antibodies
specific for each subtype. These results demonstrated an

25   increase in IgG1 class antibodies against β-amyloid in
antisera of mice that were nasally treated with 25 μg of β-
amyloid over that observed in controls. These antibodies
are associated with Th2 type cellular immune responses.

Figures 12-17 are bar graphs showing results of *in*

30   *vitro* experiments in which PLN from the PDAPP mice that were
immunized with [1-40] β-amyloid peptide prior to sacrifice
were tested by stimulation with the peptide, and tested for
cytokine production. In the animals that were mucosally
administered the [1-40] β-amyloid peptide, PLN immune

35   responses were characterized by increased production of IL-
10 and TGF-β and decreased production of IFN-γ, IL-6, and
IL-2.

In several of the experiments described above, results were obtained for administration of whole ovalbumin that were substantially different from those obtained using the other controls, and similar to results obtained using [1-40] β-amyloid. Further experiments were therefore conducted in which it was determined that whole ovalbumin is cross-reactive with [1-40] β-amyloid. Ovalbumin is therefore not an effective control in experiments designed to test immunological properties of [1-40] β-amyloid.

Example 5

Administration of β-amyloid In Conjunction with IL-4 or IL-10

Experiments were conducted in which [1-40] β-amyloid was nasally administered to (B6D2)F1 mice in conjunction with nasal administration of IL-4 or IL-10, and prior to immunization of the mice with [1-40] β-amyloid. Specifically, the (B6D2)F1 mice were nasally treated with the following compositions:

1)   PBS;

2)   [1-40] β-amyloid;

3)   IL-4;

4)   [1-40] β-amyloid+IL-4;

5)   IL-10; or

6)   [1-40] β-amyloid+IL-10

The [1-40] β-amyloid was administered in a dose of 25μg and the IL-4 and IL-10 administered in a dose of 1μg. The compositions were administered three times, at intervals of two days. Two days after the last treatment the mice were immunized with 100μg [1-40] β-amyloid in 50μg CFA. Two weeks later splenocytes from the mice were examined for proliferation and cytokine production in the presence of 50μg/ml [1-40] β-amyloid *in vitro*.

Results from these experiments are shown in Figures 18-22. In these experiments, administration of IL-4 was shown to be particularly effective in enhancing suppressive responses induced by β-amyloid peptide administration, as shown, for example in suppression of IFN-γ production, increased TGF-β production, and increased proliferative responses. The substantial decrease in IL-6 production induced by IL-4 administration in conjunction with β-amyloid peptide is relevant as increased IL-6 production is correlated with Alzheimer's disease.

Example 6

Human Treatment

An individual afflicted with Alzheimer's disease
is first orally or nasally administered 0.05 mg of [1-40] β-
5   amyloid peptide once a day for a period of one to two weeks
at the end of which the patient's cytokine responses are
measured using ELISA techniques as described above. If no
improvement is observed, the daily dosage is increased
(progressively) to 0.1 mg, 0.5 mg, 1 mg, 2.5 mg, 5 mg, 50mg
10   or 100 mg, etc. (and the cytokine responses are monitored
weekly or every two weeks) until an effective dosage is
determined. (The number of daily doses may also be
progressively increased up to six daily either instead of or
in addition to an increase in total daily amount of antigen
15   administered.) Once an effective amount and administration
schedule has been identified for this patient (over the
course of no more than several weeks or longer), therapy
continues at this amount and schedule for as long as is
beneficial. Periodically, tests of cognitive skill are
20   administered to monitor progress.

Example 7

Prevention of Alzheimer's Disease in Humans

An individual is determined to be at risk for
contracting Alzheimer's disease by examination of the
25   individual's family history of the disease, and by analysis
of magnetic resonance, positron emission tomography, SPECT
gamma camera imaging, and/or other imaging of the brain in
conjunction with suitable image enhancement agents presently
available. In this manner, the presence of amyloid plaque
30   formation in the brain is observed. In addition, the size
of the hippocampus is measured and compared with hippocampal
size typically seen in development of Alzheimer's disease.
These measures provide a reasonable prognosis for increased
risk of developing Alzheimer's disease.
35   An individual at risk for contracting Alzheimer's
disease is first orally or nasally administered 0.05 mg of
[1-40] β-amyloid peptide once a day for a period of one to

two weeks at the end of which the patient's cytokine and antibody responses are measured using ELISA techniques as described above. If no beneficial effect is observed, the daily dosage is increased (progressively) to 0.1 mg, 0.5 mg,

5   1 mg, 2.5 mg, 5 mg, 50mg or 100 mg, etc. (and the responses are monitored weekly or every two weeks) until an effective dosage is determined. (The number of daily doses may also be progressively increased up to six daily either instead of or in addition to an increase in total daily amount of

10   antigen administered.) Once an effective amount and administration schedule has been identified for this patient (over the course of no more than several weeks), therapy continues at this amount and schedule for at least 6 months. Periodically, tests of cognitive skill are administered to

15   monitor symptoms of disease.

      The invention has been described above by reference to preferred embodiments. It will be understood that various modifications are possible within the scope of the claims that follow. All documents, patents and patent

20   applications referred to in the specification are incorporated by reference in their entirety. In case of conflict, the present disclosure including the present definitions will control.

What is claimed is:

1.   A method for treating Alzheimer's disease or
suppressing the onset of said disease comprising mucosally
administering an effective amount of a composition
comprising [1-40] ß-amyloid peptide or an effective fragment
thereof.

2.   The method of claim 1 wherein the mammal is a
human.

3.   A pharmaceutical formulation for
administration to a mammal suffering from Alzheimer's
disease adapted for mucosal administration and comprising an
oral dosage form containing an effective amount of a
polypeptide comprising an amino acid sequence of [1-40] ß-
amyloid peptide, said sequence being greater than 10 amino
acids in length, and sufficient to achieve at least one of
the following:

    (i)   reduce the quantity of one or more
            proinflammatory Th1 cytokines in the
            mammal;

    (ii)   increase the quantity of one or more
            anti-inflammatory Th2 or Th3 cytokines
            in the mammal; or

    (iii)   ameliorate, inhibit or delay the onset
            of at least one clinical symptom of the
            disease.

4.   The pharmaceutical formulation of claim 3
wherein the oral dosage form is a solid dosage form selected
from the group consisting of a tablet, a capsule and a
caplet.

5.   The pharmaceutical formulation of claim 3
wherein the oral dosage form comprises an aqueous suspension
solution of ß-amyloid peptide.

6.   The pharmaceutical formulation of claim 3 further comprising a pharmaceutically acceptable carrier or diluent.

7.   A method for treating a mammal suffering from Alzheimer's disease comprising mucosally administering to the mammal an effective amount of a polypeptide comprising an amino acid sequence of β-amyloid peptide and continuing the administration for a period of time sufficient to achieve at least one of the following:

(i)   reduce the quantity of one or more proinflammatory Th1 cytokines in the mammal;

(ii)   increase the quantity of one or more anti-inflammatory Th2 or Th3 cytokines in the mammal; or

(iii)   ameliorate, inhibit or delay the onset of at least one clinical symptom of the disease.

8.   A pharmaceutical formulation adapted for mucosal administration to a mammal suffering from Alzheimer's disease comprising an aerosol dosage form containing an effective amount of a polypeptide comprising an amino acid sequence of [1-40] ß-amyloid peptide, said sequence being greater than 10 amino acids in length, and sufficient to achieve at least one of the following:

(i)   reduce the quantity of one or more proinflammatory Th1 cytokines in the mammal;

(ii)   increase the quantity of one or more anti-inflammatory Th2 or Th3 cytokines in the mammal; or

(iii)   ameliorate, inhibit or delay the onset of at least one clinical symptom of the disease.

9.   The pharmaceutical formulation of claim 8
further comprising a pharmaceutically acceptable carrier or
diluent.

      10.   A method for preventing Alzheimer's disease
in a mammal at risk for contracting the disease comprising
orally administering to the mammal an effective amount of a
polypeptide comprising an amino acid sequence of β-amyloid
peptide and continuing the administration for a period of
time sufficient to inhibit or delay onset of at least one of
the following:
            (i)   generation of one or more
                     proinflammatory Th1 cytokines in the
                     mammal;
            (ii)   decline in the quantity of one or more
                     anti-inflammatory Th2 or Th3 cytokines
                     in the mammal; or
            (iii)   appearance of at least one clinical
                     symptom of the disease.

      11.   A method for preventing Alzheimer's disease
in a mammal at risk for contracting the disease comprising
administering by inhalation to the mammal an effective
amount of a polypeptide comprising an amino acid sequence of
β-amyloid peptide and continuing the administration for a
period of time sufficient to inhibit or delay onset of at
least one of the following:
            (i)   generation of one or more
                     proinflammatory Th1 cytokines in the
                     mammal;
            (ii)   decline in the quantity of one or more
                     anti-inflammatory Th2 or Th3 cytokines
                     in the mammal; or
            (iii)   appearance of at least one clinical
                     symptom of the disease.

      12.   A pharmaceutical formulation adapted for
mucosal administration to a mammal at risk for contracting
Alzheimer's disease comprising an effective amount of a

polypeptide comprising an amino acid sequence of β-amyloid peptide in an aerosol form sufficient to inhibit or delay onset of at least one of the following:

(i)   generation of one or more proinflammatory Th1 cytokines in the mammal;

(ii)   decline in the quantity of one or more anti-inflammatory Th2 or Th3 cytokines in the mammal; or

(iii)   appearance of at least one clinical symptom of the disease.

13.   The pharmaceutical formulation of claim 11 further comprising a pharmaceutically acceptable carrier or diluent.

14.   A method of treating an individual suffering from, or at risk for suffering from, Alzheimer's disease comprising administering an effective amount of a composition comprising a peptide comprising the amino acid sequence of a fragment of ß-amyloid peptide or an analog thereof effective to suppress immune response associated with said Alzheimer's disease.

15.   The method of claim 14 comprising oral administration.

16.   The method of claim 15 comprising administration by inhalation.

17.   The method of claim 14 comprising administration of ß-amyloid peptide.

18.   The method of claim 14 wherein said peptide fragment comprises a homologous, conservative, non-peptidic or isosteric replacement for one or more amino acids of ß-amyloid peptide.

19. A method of treating Alzheimer's disease
comprising administering an effective amount of an analog or
mimetic of ß-amyloid peptide or of an effective fragment
thereof to an individual suffering from said disease.

5   20. A method for treating Alzheimer's disease or
suppressing the onset of said disease comprising mucosally
administering an effective amount of a peptide composition
comprising an amino sequence of β-amyloid protein effective
to suppress said disease, wherein said peptide composition
10   is in a daily dose greater than 0.05 mg.

21. The method of claim 20 wherein said
administration induces production of anti-β-amyloid
antibodies.

22. The method of claim 21 wherein said
15   antibodies are of the IgG1 type.

23. The method of claim 20 wherein said
administration comprises oral administration that delivers
said peptide composition primarily to the gastric mucosa.

24. The method of claim 23 wherein less than 10%
20   of said peptide composition is released in the mouth from a
dosage form employed for said administration.

25. The method of claim 20 wherein said
administration comprises nasal administration.

FIG. IA

FIG. IB

FIG. 2A

FIG. 2B

FIG. 3A

FIG. 3B

FIG. 4A

FIG. 4B

FIG. 5A

Bar chart: y-axis "IL-2 PG/ML" from 0 to 400. x-axis "TREATMENT" with categories NONE, FED 500, FED 50, FED 5, NASAL 50, NASAL 5.

FIG. 5B

Bar chart: y-axis "IL-2 SP PG/ML" from 0 to 150. x-axis "TREATMENT" with categories NONE, FED 500, FED 50, FED 5, NASAL 50, NASAL 5.
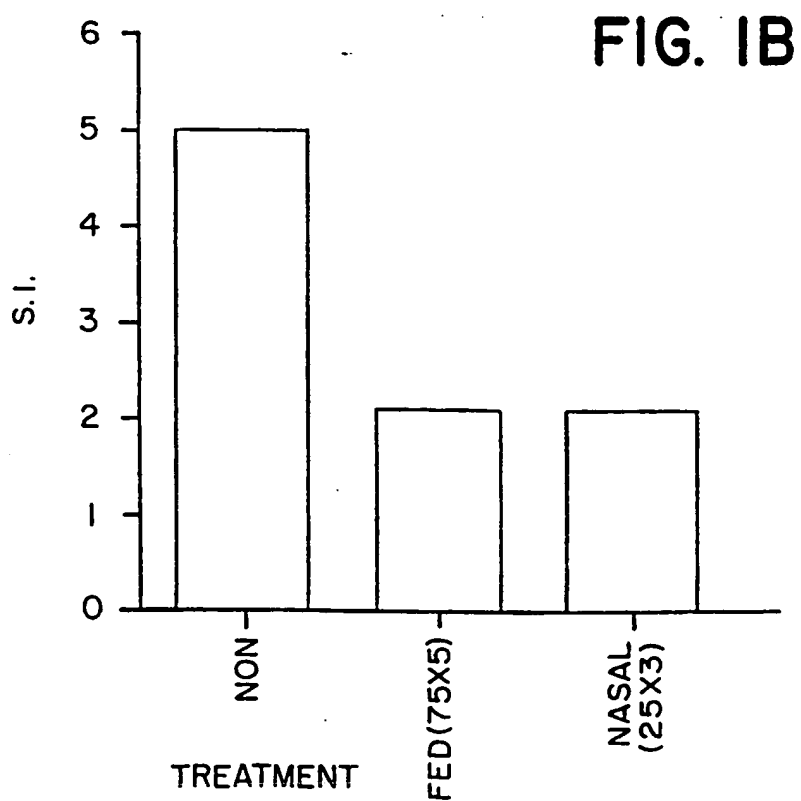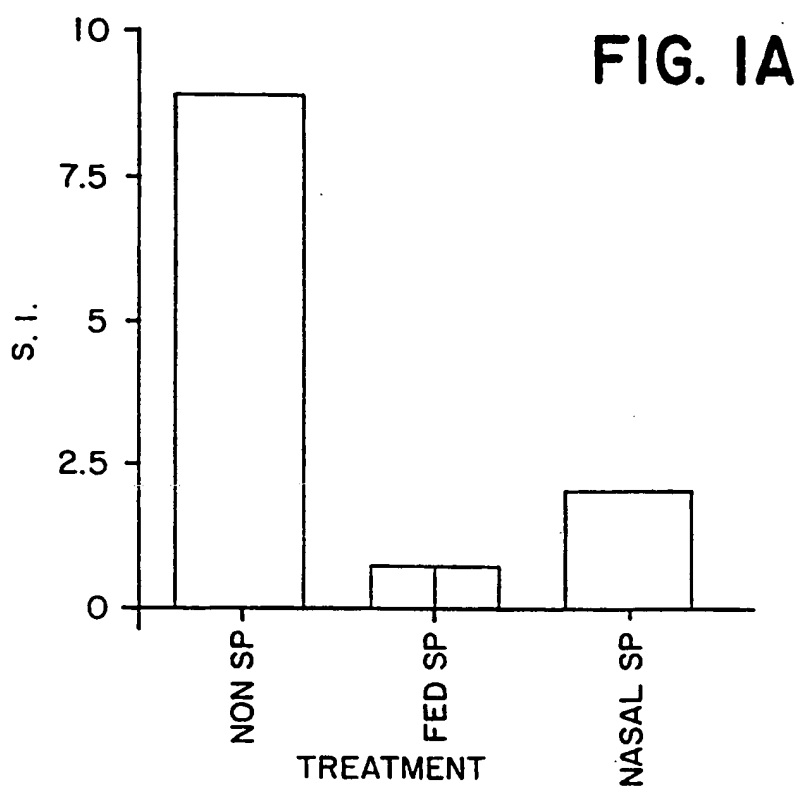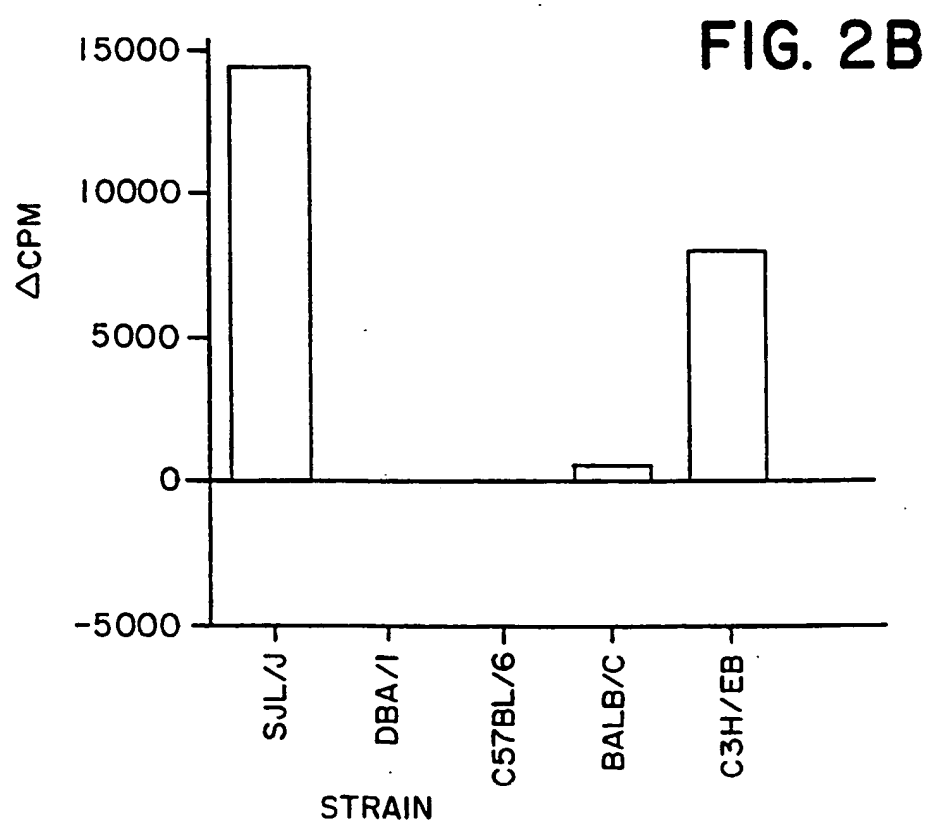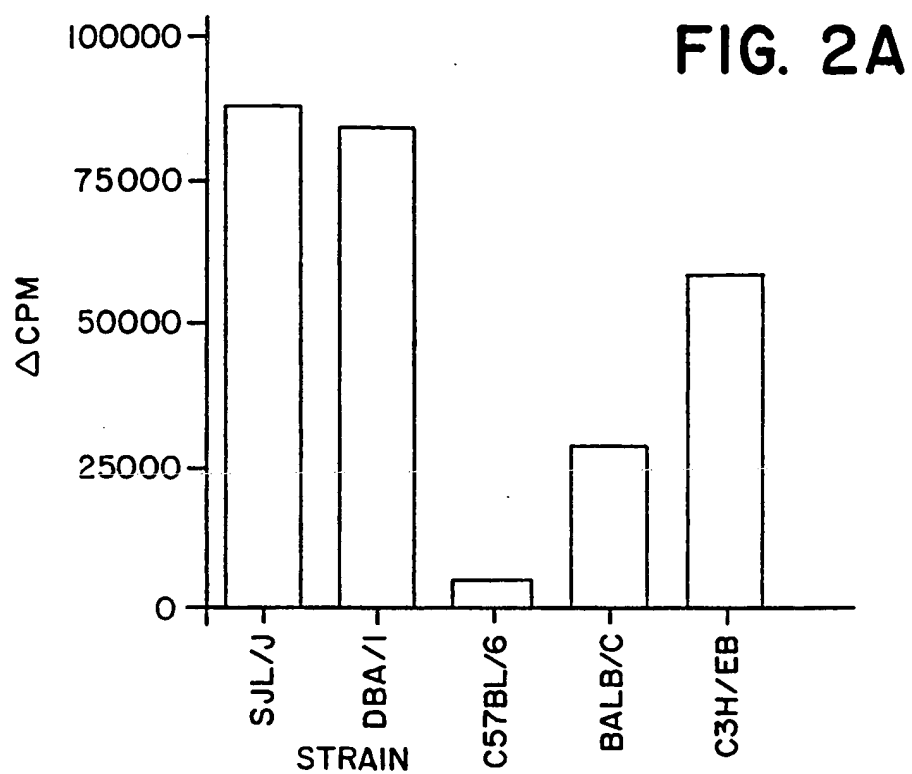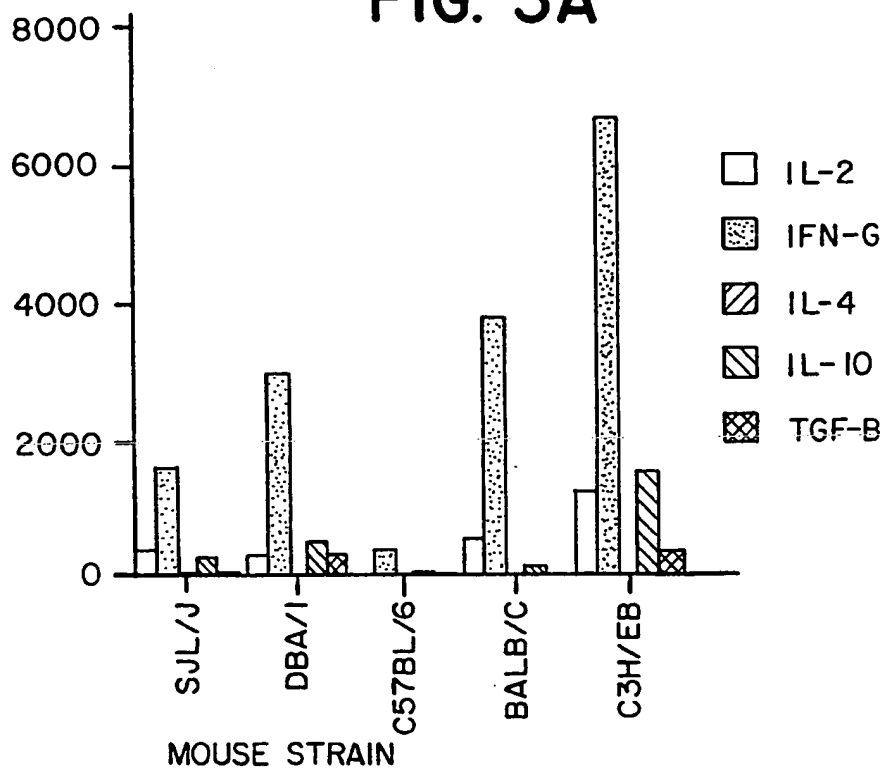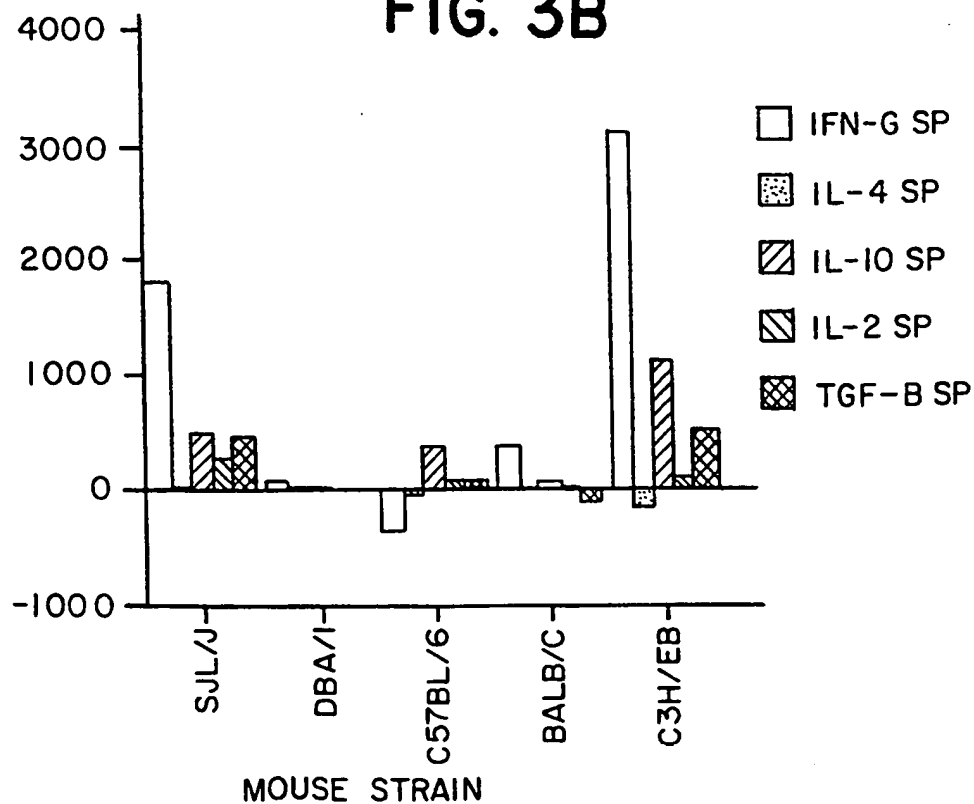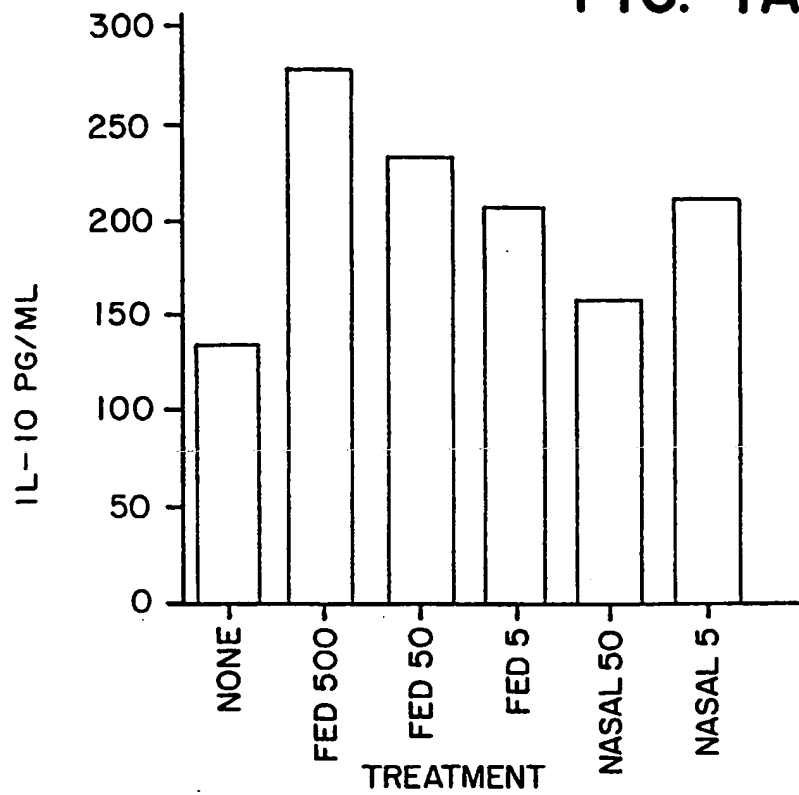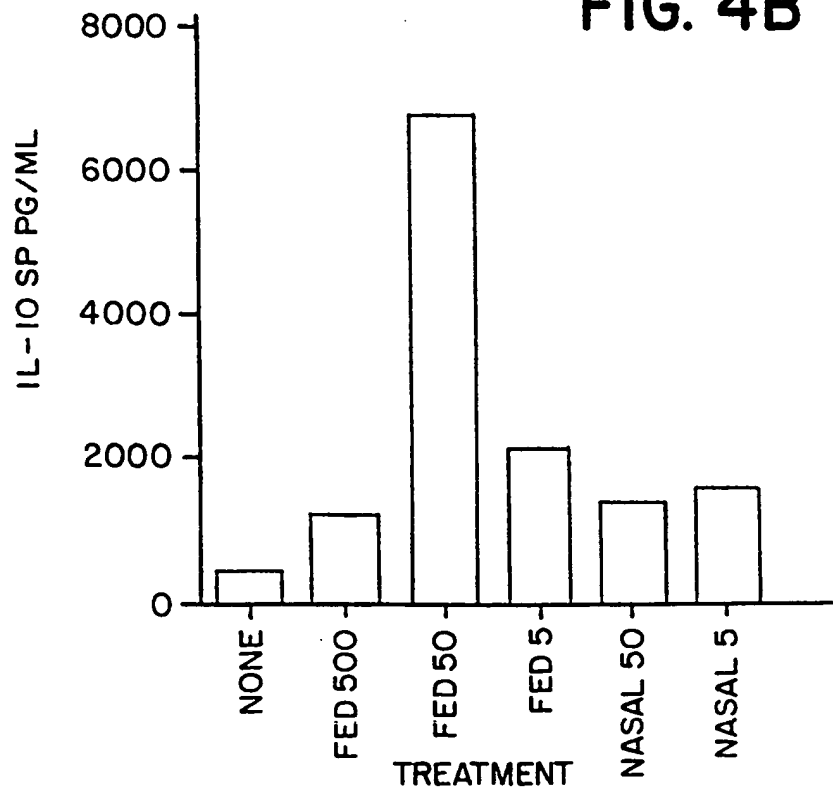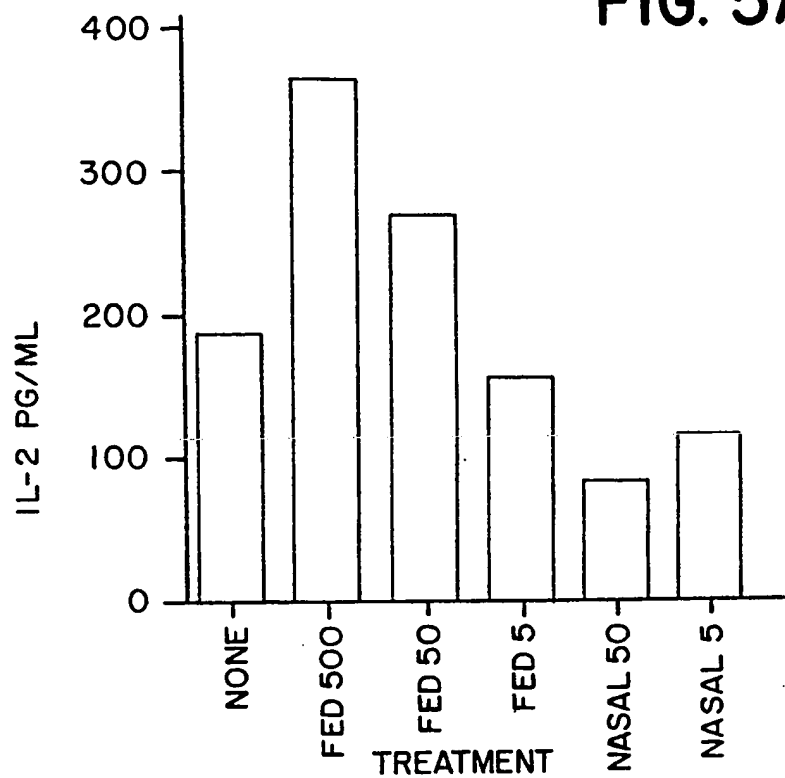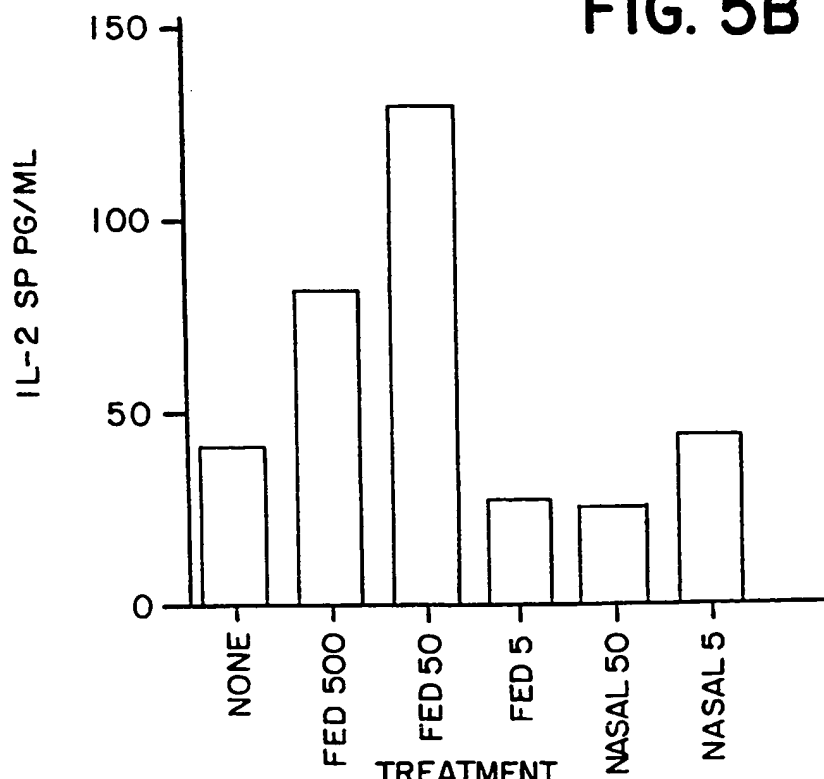
FIG. 6A
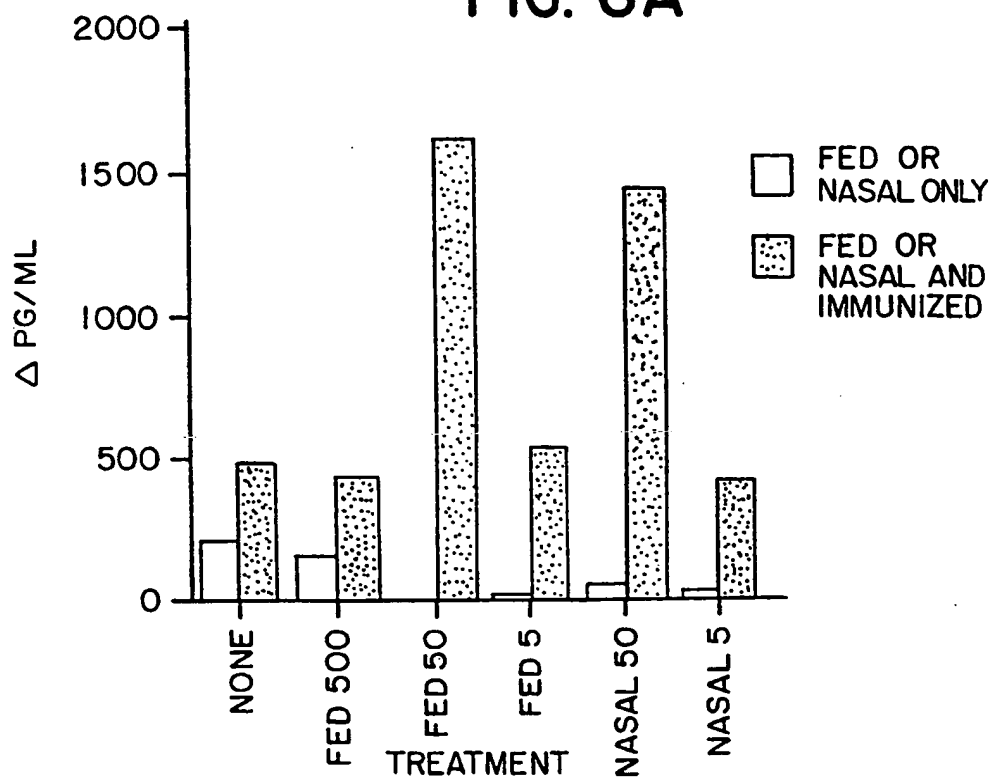
FIG. 6B
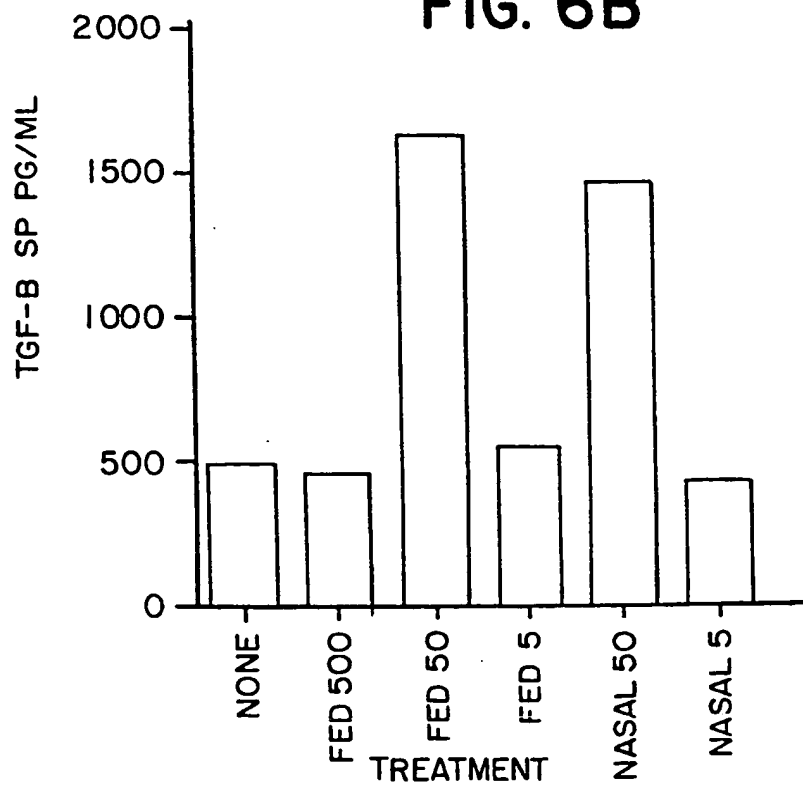
FIG. 7A
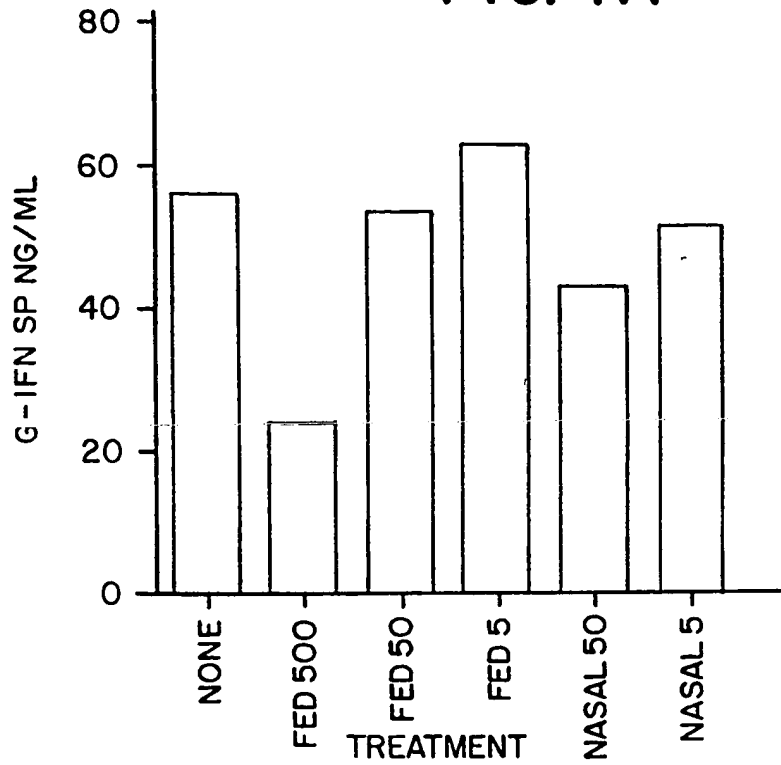
FIG. 7B
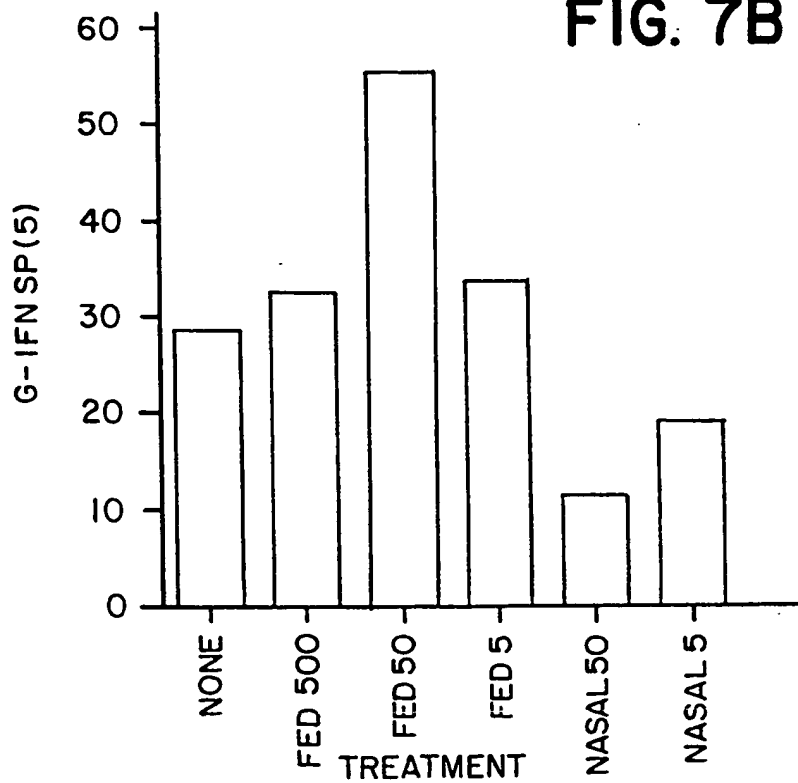
FIG. 8A
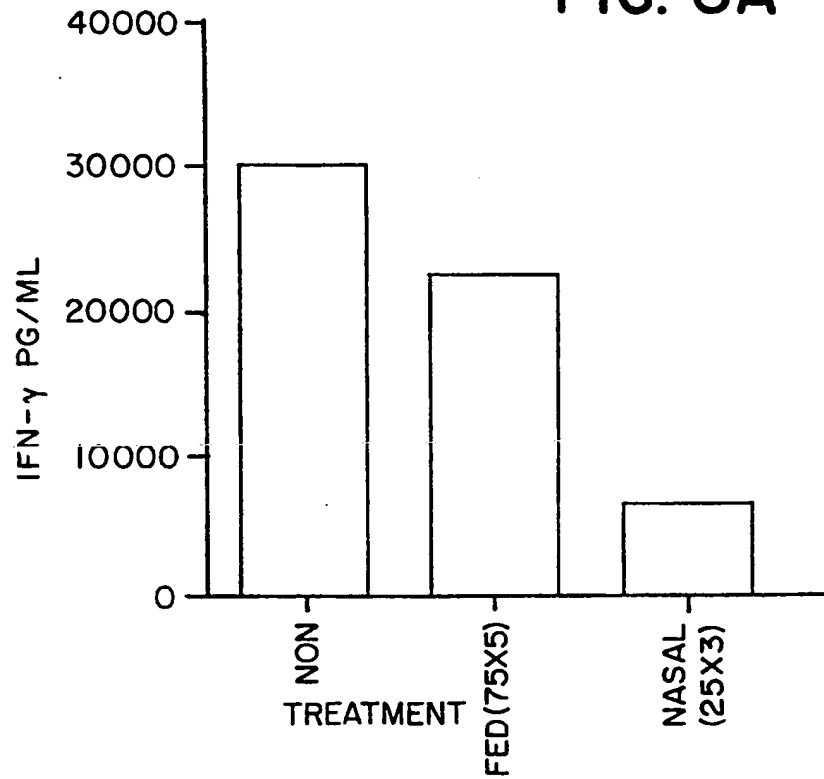
FIG. 8B
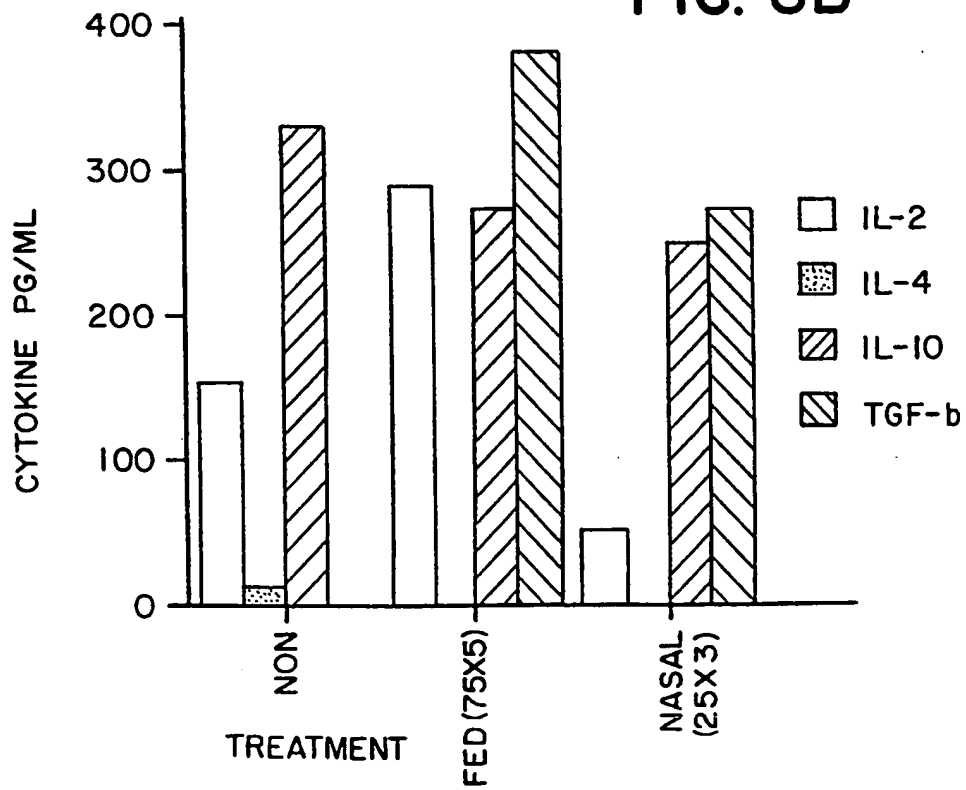
64 PDAPP Mice   Fig. 9A

PAGE 1

Experimental Group		AB Load: % Area (computer)	AB Load (Visual) a	b							
				Mac-1	F4/80	MHCII	IFN-γ	TNF-α	IL-4	IL-10	TGF-β
AB 100 µg oral	1.1	0.0466	0	-	-	-	-	-	+	+	-
	1.2	0.50	4								
	1.3	1.0	4	-	-	-	-	-	+	-	-
	1.4	0.44	3								
	1.5	0.12	1.5	+	+	+	-	-	+	-	-
	1.6	3.24	4.5								
	1.7	3.59	5	-	-	-	+	+	+	-	-
	1.8	2.36	6								
	1.9	0.18	1	-	+	-	-	-	+	++	-
AB 10 µg oral	2.1	-0-	-0-	-	-	-	-	-	-	+	-
	2.2	1.04	4								
	2.3	0.0267	0.5	-	-	-	-	-	+	-	-
	2.4	0.32	3								
	2.5	1.76	5	-	-	-	-	-	-	-	-
	2.6	3.87	6								
	2.7	0.90	4	-	-	-	-	-	+	-	-
	2.8	0.46	3								
	2.9	0.85	3.5	-	-	-	-	-	-	-	-
AB 25 µg nasal	3.1	0.23	2	-	-	-	-	-	+	+	-
	3.2	0.0921	0.5								
	3.3	1.36	4.5	-	-	-	-	-	+	-	-
	3.4	0.48	3								
	3.5	0.30	3	-	-	-	-	-	-	-	-
	3.6	0.71	3								
	3.7	0.34	1	-	-	-	-	-	+	-	-
	3.8	0.59	2.5								
	3.9	0.41	1	-	-	-	-	-	+	-	-
AB 5 µg nasal	4.1	0.38	2.5	-	-	-	-	-	+	-	-
	4.2	1.93	5								
	4.3	0.00236	0	-	-	-	-	-	-	-	-
	4.4	0.0461	0.5								
	4.5	1.61	3.5	-	-	-	-	-	+	-	-
	4.6	0.37	2.5								
	4.7	1.72	5	-	-	-	-	-	+	-	-

a	Scoring
0 = none	
0.5 = 1 to 4 isolated plaques	
1 = 5 to 10 isolated plaques	
2 = mild	
3 = moderate	
4 = moderately severe	
5 = severe	

b	
-	no immunoreactivity (IR)
+	1-10 IR cells
++	10-100 IR cells
+++	> 100 IR cells

64 PDAPP Mice

Fig. 9B

PAGE 2

Experimental Group		AB Load: % Area (computer)	AB Load (Visual)a	b							
				Mac-1	F4/80	MHCII	IFN-γ	TNF-α	IL-4	IL-10	TGF-β
non-treated	0.1	0.64	3.5	+	+	+	+	+	++	++	-
	0.2	1.33	3.5								
	0.3	0.0761	1	+	-	-	+	+	+	+	-
	0.4	1.31	4.5								
	0.5	0.29	2	-	-	-	++	++	++	-	-
	0.6	3.59	4.5								
	0.7	0.72	3	+	+	+	++	++	-	-	-
OVA 500 µg oral	5.1	0.42	3	-	-	-	-	-	+	+	-
	5.2	0.71	3.5								
	5.3	0.89	4	-	-	-	-	-	+	-	-
	5.4	0.48	3								
	5.5	2.96	4	++	++	++	+	++	+	++	-
	5.6	1.28	3.5								
OVA 50 µg nasal	6.1	0.17	2	++	++	+	-	-	+	+	-
	6.2	0.0682	0.5								
	6.3	0.65	4	-	-	-	-	-	+	-	-
	6.4	0.17	1.5								
	6.5	1.41	3.5	+	+	-	+	+	+	+	-
	6.6	1.97	0.5								
MBP 500 µg oral	7.1	0.90	4.5	++	++	++	-	-	++	+	-
	7.2	0.87	4.5								
	7.3	1.09	4	+	+	-	+	+	+	-	-
	7.4	2.44	5								
	7.5	3.76	5	++	+	+	+	+	+	-	-
MBP 50 µg nasal	8.1	0.24	2	++	++	++	+	+	+	+	-
	8.2	1.03	4								
	8.3	0.32	3	+	+	++	+	+	-	-	-
	8.4	1.20	4.5								
	8.5	4.07	5	++	++	+	+	+	-	++	-
	8.6	0.27	1								

a	Scoring
	0 = none
	0.5 = 1 to 4 isolated plaques
	1 = 5 to 10 isolated plaques
	2 = mild
	3 = moderate
	4 = moderately severe
	5 = severe

b	
-	no immunoreactivity (IR)
+	1-10 IR cells
++	10-100 IR cells
+++	> 100 IR cells

Aß burden
Image analysis – HC and Cortex
1-tailed Mann-Whitney U-test

Fig.10

I. Statistically Significant Results

<u>P value</u>

Grp	3		vs	Grps	0, 5, 6, 7, 8		0.0462
Grp	3		vs	Grps	0, 7, 8		0.0380
Grp	3		vs	Grps	7, 8		0.0337

II. <u>Not Quite</u> Statistically Significant Results

Grps	1, 2, 3, 4	vs	Grps	0, 5, 6, 7, 8	0.0937
Grps	1, 2, 3, 4	vs	Grps	0, 7, 8	0.0719
Grps	1, 2, 3, 4	vs	Grps	7, 8	0.0645
Grp	6	vs	Grps	7, 8	0.0901
Grps	3, 4	vs	Grps	0, 7, 8	0.0583
Grps	3, 4	vs	Grps	0, 5, 6, 7, 8	0.0598
Grps	3, 4	vs	Grps	7, 8	0.0544

III. <u>Not</u> Statistically Significant

Grp	6	vs	Grps	0, 7, 8	0.1254
Grps	5, 6	vs	Grps	7, 8	0.1550
Grps	5, 6	vs	Grps	0, 5, 6, 7, 8	0.2840
Grps	3, 4	vs	Grps	0, 6, 8	0.2811
Grp	3	vs	Grps	0, 6, 8	0.2303
Grp	6	vs	Grps	1, 2, 3, 4	0.4028
Grp	8	vs	Grp	0	0.1496
Grp	3	vs	Grp	1, 2	0.1981

Fig.11

ral Tolerance: Immunohistochemistry of Down's Syndrome Brain Sections using PDAPP mouse antisera

Each section was pre-treated with formic acid and IgG isotype specific antibodies were used to detect IgG_1, IgG_{2a} and IgG_{2b} produced against $A\beta$.

Mouse antisera (1:10 dilution)	General IgG 2^0 Ab Plaque staining	IgG_1 2^0 Ab Plaque staining	IgG_{2a} 2^0 Ab Plaque staining	IgG_{2b} 2^0 Ab Plaque staining
$A\beta$ oral 100μg 1.9*	+	+ •	-	- +
$A\beta$ oral 10μg 2.7	+	•	-	-
2.9	++	+	+	+
$A\beta$ nasal 25μg 3.1	++	+	-	+
3.2	++	++ •	-	-
3.3	++	+	-	+
3.4	++	+	-	+
3.5	-	-	-	-
3.6	+	+	-	+
3.7	+ •	+	-	+
3.8	•	+	-	-
3.9	+	+	-	+
$A\beta$ nasal 5μg 4.1	+	•	-	•
4.2	•	•	-	•
4.3	++	-	-	•
4.6	+	+	+	+
4.7	+	+	-	-
OVA oral 500μg 5.5* IgM+	+	+ •	-	+
MBP oral 500μg 7.5*	+	- •	-	•
MBP nasal 50μg 8.5*	+	+	+	-
8.6* IgM↑	+	+	-	+

- negative
+ positive: sub-population of plaques stain d or weak staining of numerous plaques
++ numerous plaques stained with strong immunoreativity

* immunized with Ab(1-40) at 12 months, waited 10 days, then sacrificed

FIG. 12
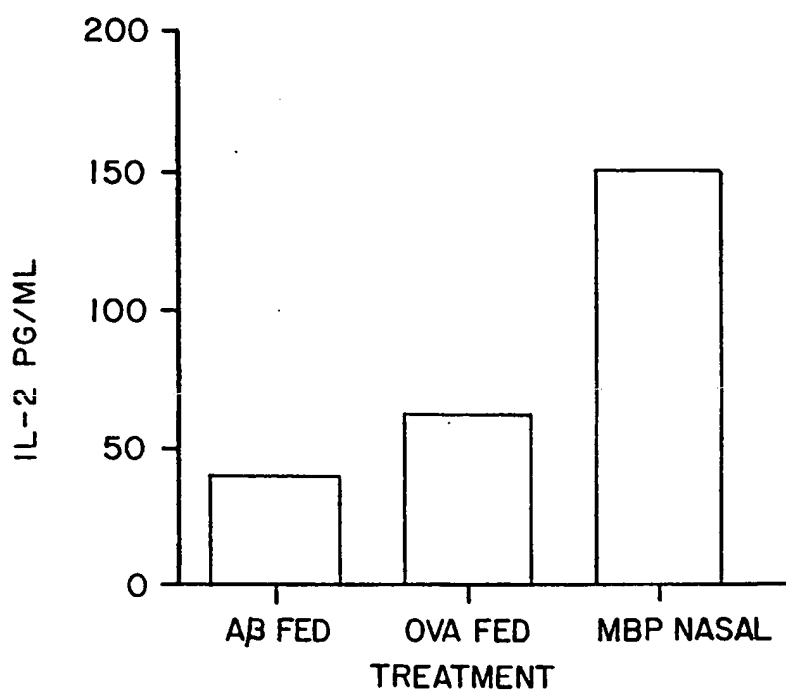
FIG. 13
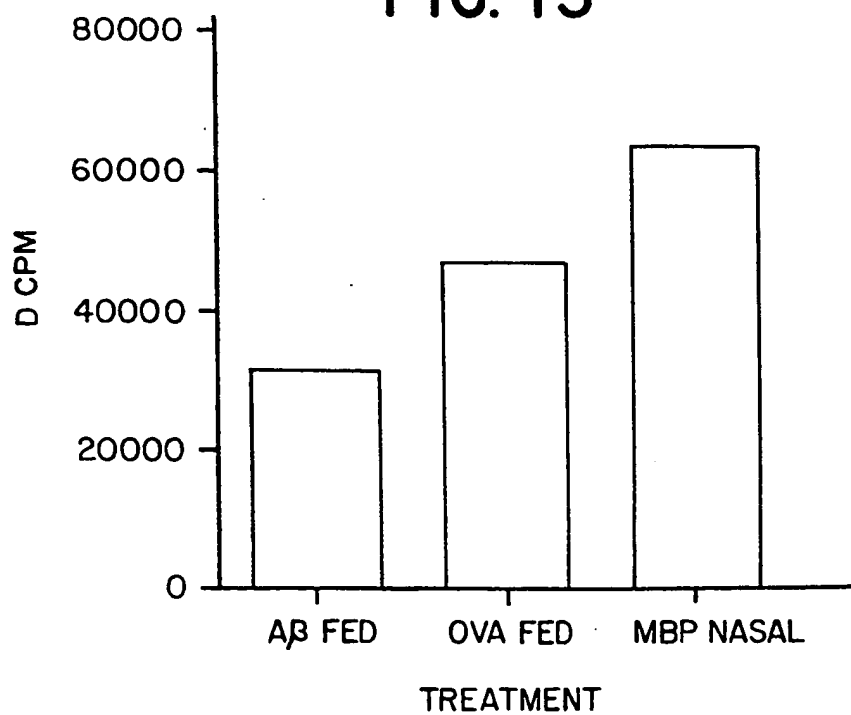
FIG. 14

FIG. 15

FIG. 16

FIG. 17

FIG. 18

TREATMENT

(bar chart with y-axis 0 to 30, treatments: PBS, Aβl-40, IL-4, Aβl-40+IL-4, IL-10, Aβl-40+IL-10)

FIG. 19

TREATMENT

(bar chart with y-axis 0 to 25, treatments: PBS, Aβl-40, IL-4, Aβl-40+IL-4, IL-10, Aβl-40+IL-10)

FIG. 20

FIG. 21

FIG. 22

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/17
US CL :514/12, 885
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 885

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155, 351, 357, 358) search terms: Alzheimer's, amyloid, mucosal, oral tolerance, inflammation, cytokine, nasal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96/39176 A1 (BRIGHAM & WOMEN'S HOSPITAL) 12 December 1996 (12.12.96), see entire document.	1-25
A, P	TANAKA et al. NC-1900, an active fragment analog of arginine vasopressin, improves learning and memory deficits induced by beta-amyloid protein in rats. European Journal of Pharmacology. 1998, Vol. 352, pages 135-142, especially abstract.	1-25
A	GOZES et al. Neuroprotective strategy for Alzheimer disease: Intranasal administration of a fatty neuropeptide. Proceedings of the National Academy of Sciences. January 1996, Vol. 93, pages 427-432, especially abstract.	1-25

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 FEBRUARY 1999	**17 FEB 1999**

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| C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FINCH et al. Evolutionary Perspectives on Amyloid and Inflammatory Features of Alzheimer Disease. Neurobiology of Aging. 1996, Vol. 17, No. 5, pages 809-815, see entire document.	1-25
A	TRIEB et al. Is Alzheimer beta amyloid precursor protein (APP) an autoantigen? Peptides corresponding to parts of the APP sequence stimulate T lymphocytes in normals, but not in patients with Alzheimer's disease. Immunobiology. 1994, Vol. 191, No. 2-3, page 114-115, abstract C.37, see entire document.	1-25

Fig. 12

IL-2 PRODUCTION IN PLN FROM PDAPP MICE FED WITH Aβ 1-40,
OVALBUMIN OR M. BP PRIOR TO IMMUNIZATION WITH Aβ 1-40.
(50ug Aβ 1-40 in vitro).
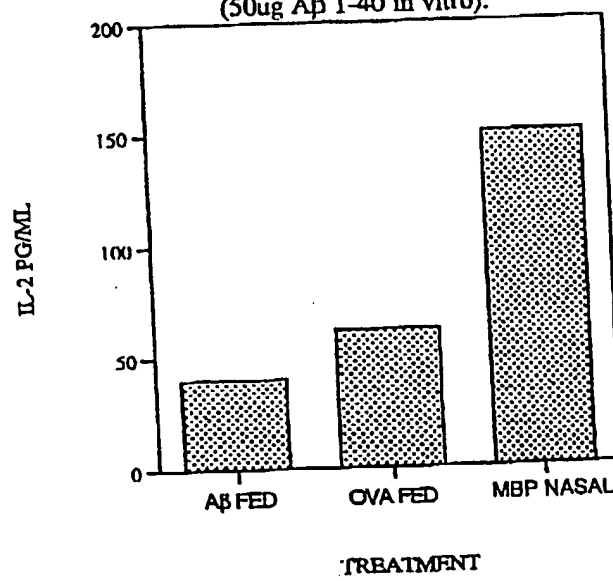
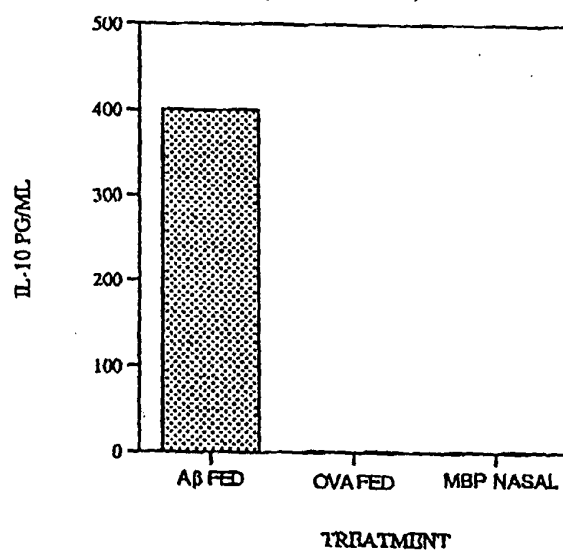Fig. 13

PROLIFERATION OF PLN FROM PDAPP MICE FED WITH Aβ 1-40,
OVALBUMIN OR MBP PRIOR TO IMMUNIZED WITH Aβ 1-40.
(50ug Aβ 1-40 in vitro).
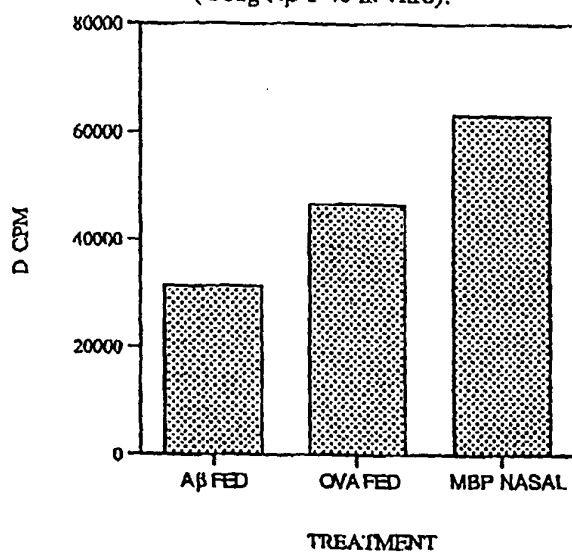
TREATMENT

Fig. 14

IFN-γ PRODUCTION IN PLN FROM PDAPP MICE FED WITH Aβ 1-40,
OVALBUMIN OR MBP PRIOR TO IMMUNIZATION WITH Aβ 1-40.
(50ug Aβ 1-40 in vitro).

TREATMENT
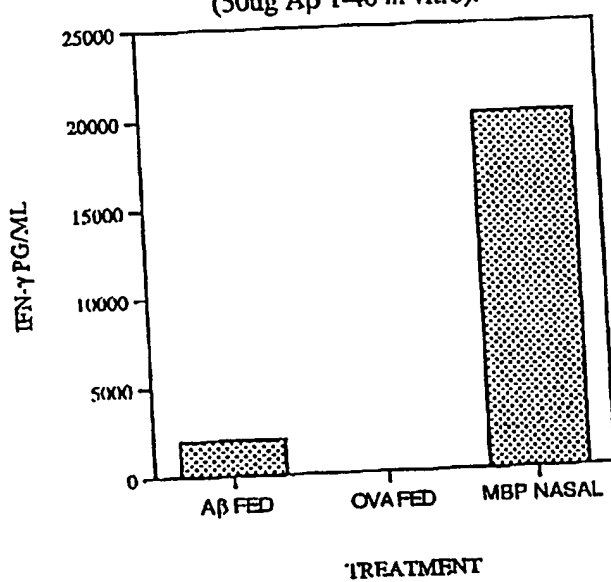
Fig. 15

IL-6 PRODUCTION IN PLN FROM PDAPP MICE FED WITH Aβ 1-40.
OVALBUMIN OR MBP PRIOR TO IMMUNIZATION WITH Aβ 1-40.
(50ug Aβ 1-40 in vitro)

TREATMENT
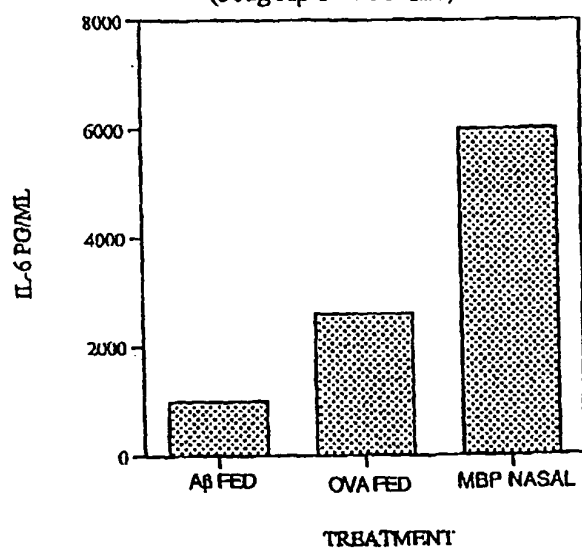
Fig. 16

IL-10 PRODUCTION IN PLN FROM PDAPP MICE FED WITH Aβ 1-40,
OVALBUMIN OR MBP PRIOR TO IMMUNIZATION WITH Aβ 1-40.
(50ug Aβ 1-40 in vitro).

Fig.17

TGF-β PRODUCTION IN PLN FROM PDAPP MICE FED WITH Aβ 1-40,
OVALBUMIN OR MBP PRIOR TO IMMUNIZATION WITH Aβ 1-40.
(50ug Aβ in vitro).
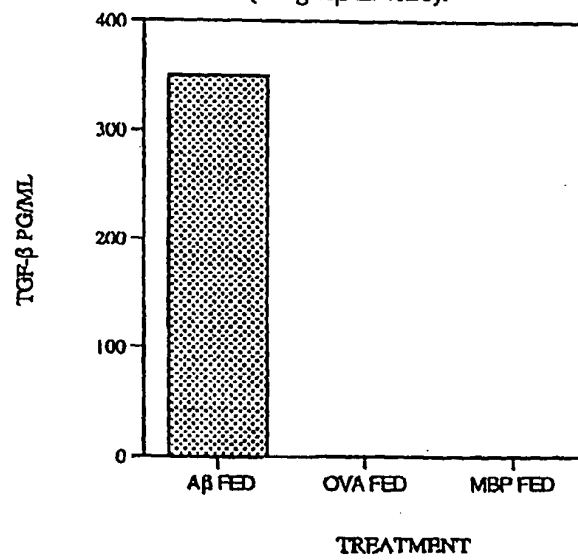
Fig. 18

PROLIFERATION OF SPLEENS FROM (B6D2)F1 MICE NASALLY
TREATED WITH: PBS, Ab 1-40, IL-4, Ab+IL-4, IL-10 AND Ab+IL-10
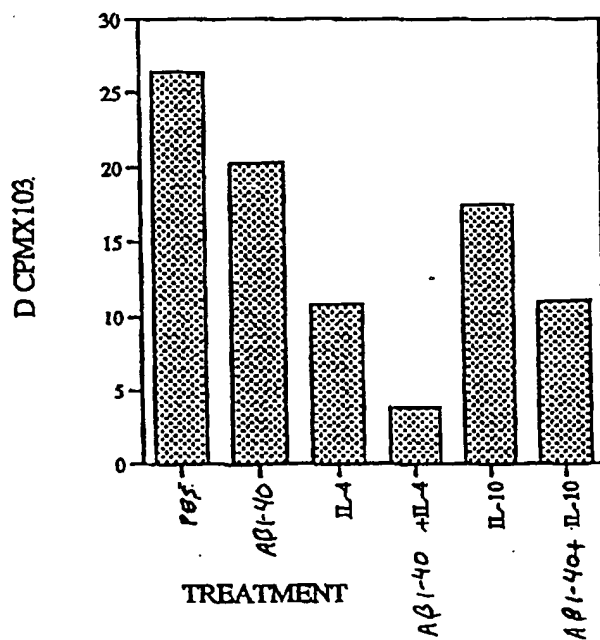PRIOR TO IMMUNIZATION WITH Ab 1-40.

Fig.19

IFN-γ PRODUCTION IN SPLEENS FROM (B6D2)F1 MICE NASALLY
TREATED WITH: PBS, Ab 1-40, IL-4, Ab+IL-4, IL-10 AND Ab+IL-10
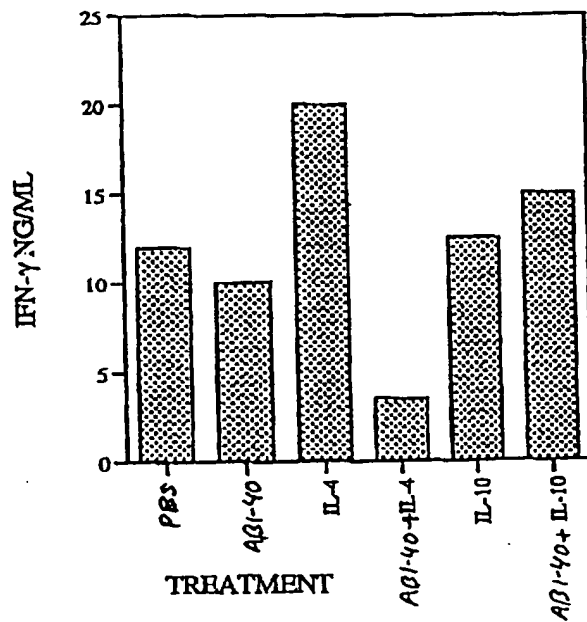PRIOR TO IMMUNIZATION WITH Ab 1-40,

Fig. 20

IL-6 PRODUCTION IN SPLEENS FROM (B6D2)F1 MICE NASALLY
TREATED WITH: PBS, Ab 1-40, IL-4, Ab+IL-4, IL-10, AND Ab+IL-10
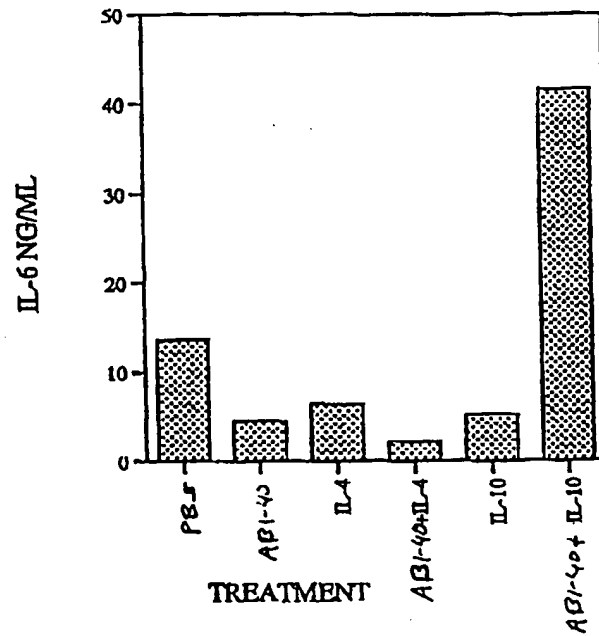PRIOR TO IMMUNIZATION WITH Ab 1-40.

Fig. 21

IL-10 PRODUCTION IN SPLEENS FROM (B6D2)F1 MICE NASALLY
TREATED WITH: PBS, Ab 1-40, IL-4, Ab+IL-4, IL-10 AND Ab +IL-10
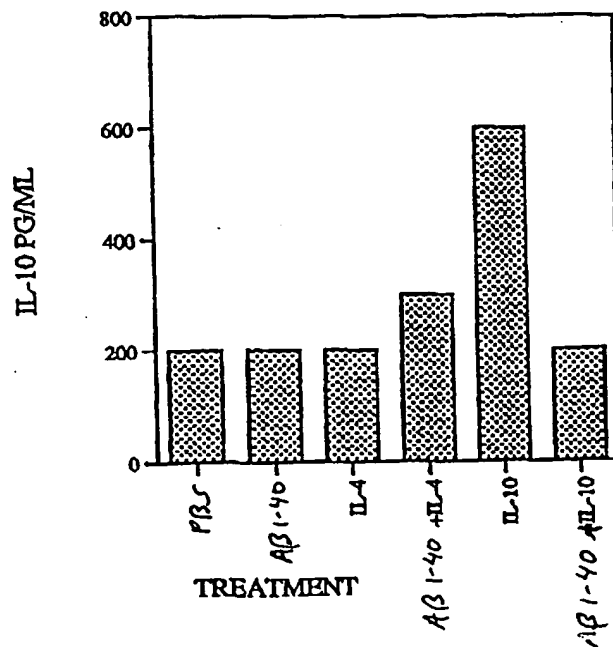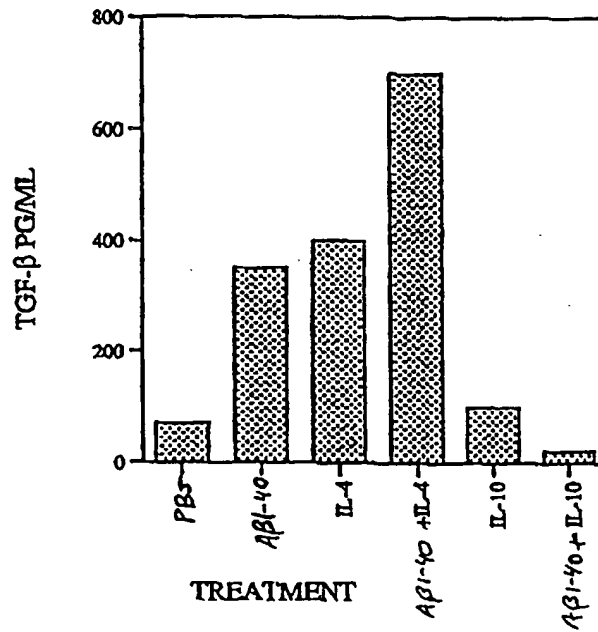PRIOR TO IMMUNIZATION WITH Ab 1-40.

Fig. 22

TGF-β PRODUCTION IN SPLEENS FROM (B6D2)F1 MICE NASALLY
TREATED WITH:PBS, Ab1-40, IL-4, Ab+IL-4, IL-10 AND Ab+IL-10
PRIOR TO IMMUNIZATION WITH Ab 1-40.

INTERNATIONAL SEARCH REPORT

A.   CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :A61K 38/17
US CL   :514/12, 885

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"E" earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 FEBRUARY 1999	**17 FEB 1999**
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer MARIANNE P. ALLEN
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	TRIEB et al. Is Alzheimer beta amyloid precursor protein (APP) an autoantigen? Peptides corresponding to parts of the APP sequence stimulate T lymphocytes in normals, but not in patients with Alzheimer's disease. Immunobiology. 1994, Vol. 191, No. 2-3, page 114-115, abstract C.37, see entire document.	1-25